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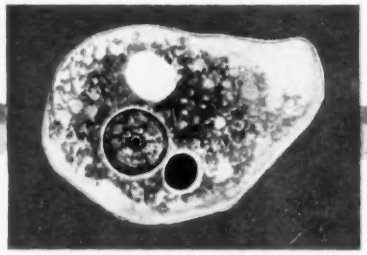

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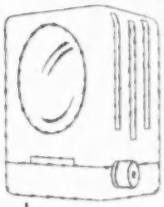

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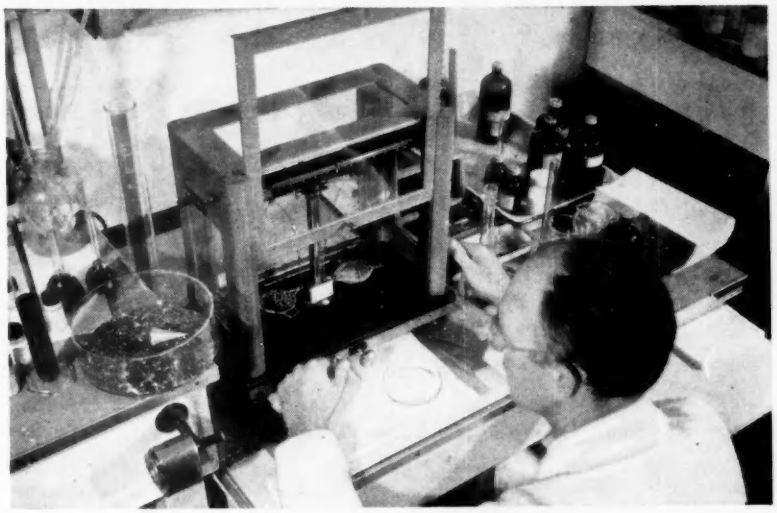

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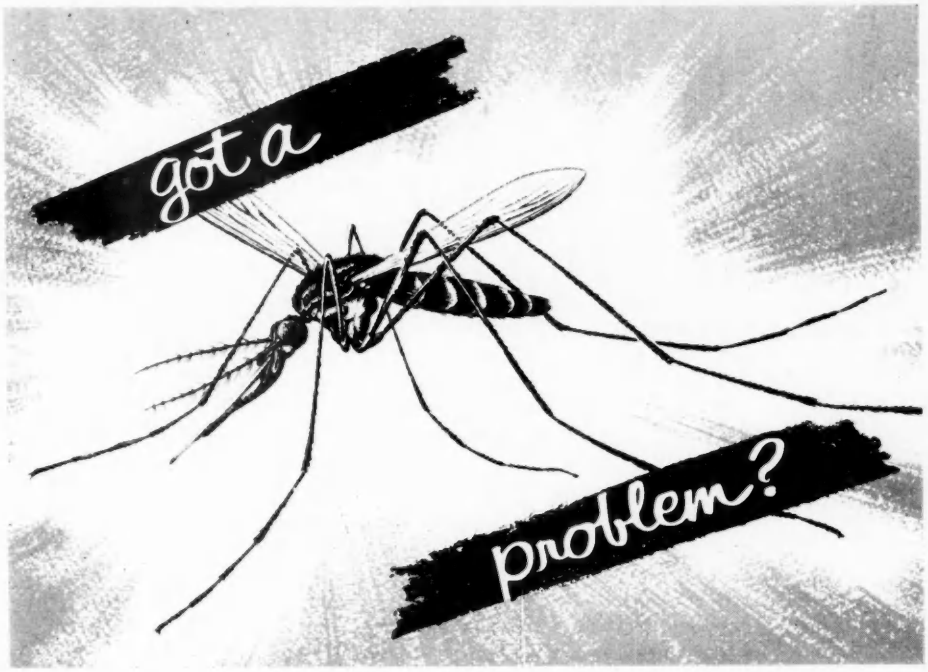

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HEMORRHAGIC FEVER. I. EPIDEMIOLOGY

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Early in June, 1951, members of the United Nations Forces in Korea entered medical evacuation channels with chills and fever, severe retrobulbar headache, generalized myalgia and joint pains, severe vomiting, albuminuria, leucocytosis, and conjunctival hemorrhages. Diagnosis in these early cases included thrombocytopenic purpura, hemorrhagic diathesis, blood dyscrasia (thrombocytopenia, virus hemorrhagic disease, bacteremia, acute leukemia and infectious mononucleosis), acute nephritis and Weil's disease.

Darkfield examination of blood from some of the first patients showed filamentous forms which were erroneously classified as leptospiras. Enright and Fennel (*5*) reported a similar experience which led to the erroneous diagnosis of Weil's disease. Antibody studies of acute and convalescent sera showed no titer suggestive of leptospirosis, whether CF or agglutination strains were employed (*11*).

The Japanese, as early as 1939, experienced a disease of the same symptomatology in Manchuria and in 1942 they recognized it as a disease entity, giving it the name "Epidemic Hemorrhagic Fever." In retrospect the Japanese established to their satisfaction that such disease outbreaks had occurred as early as 1935 (*4*). TB Med 208, Medical and Sanitary Data on Korea (*1*) gave a brief description, mentioning the disease as of potential military importance. Hsiao (*2*) mentioned it as being transmitted by a "tick" (Japanese—*dani*, meaning tick or mite). Craig (*3*) prepared a concise summary of the disease for medical officers in the Far East Command in February 1951. Takami (*4*) presented the historical background and a more detailed review of the Japanese findings than had previously been available in English.

The Russian literature refers to at least four virus hemorrhagic fevers, all characterized by the hemorrhagic syndrome (*14*, *15*, *16*, *17*). Russian clinical and pathological descriptions of Far Eastern nephroso-nephritis correspond most closely to the Japanese findings for epidemic hemorrhagic fever and to the disease experienced by the United Nations Forces in Korea.

The disease was practically unknown among Korean physicians. Major Y. U. Lee, M.C., R.O.K.A., who had seen cases in Mukden in 1941, visited patients in a U. S. Army Hospital and a R.O.K.A. Hospital during November, 1951, in company with the author and stated that they presented the clinical signs and

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[2] I wish to express my sincere appreciation to the officers and enlisted men of the Army Medical Service of the Eighth U. S. Army and the Far East Command who assisted in the investigation of cases and the collection and identification of rodents and their ectoparasites.

[3] This report includes data on 89 cases collected by Col. James H. Gordon, M.C., U.S.A., Chief of the Preventive Medicine Division prior to 31 August 1951.

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symptoms of patients seen by him in Manchuria. One prisoner of war, a bacteriologist from North Korea, had read Kitano's paper (*18*), but had seen no cases.

The Japanese reported this disease to be of virus etiology transmitted to man by a mite, *Laelaps jettmari* Vitzthum, with a rodent reservoir in the mouse *Apodemus agrarius*, suggesting a disease analogous to scrub typhus (*9*). The Russians reported the disease to be of virus etiology, with a flea vector and a reservoir in the vole *Microtus michnoi*.

Clinically this disease generally has an abrupt onset with a prodromal period of one or two days with malaise and grippe-like symptoms. Temperatures may rise to 105°F. and usually will have returned to normal by the end of the first week. Vomiting and severe retrobulbar headache occur early in the course of the disease. There is a leucocytosis which occurs about the third day and generally rises to over 20,000 per cu. mm. However, counts as high as 98,000 per cu. mm. have been reported. The white count generally returns to normal by the end of the second week. The hemorrhagic manifestations, which are so striking in this disease, appear about the sixth to eighth day and usually will have disappeared by the end of the third or fourth week. The scleral hemorrhages are the most striking manifestations of this disease. The urine shows albuminuria and a decrease in specific gravity, associated with an oliguria, which continues for approximately a week, and then the renal function returns to normal. Complete recovery occurs in 3–5 weeks. Death may occur up to 28 days.

Pathological findings are most striking in the kidney and the pituitary. The medulla of the kidney is dark red and hemorrhagic while the cortex is edematous and pale. The pituitary shows extensive focal necrosis. Hemorrhages may be found in any organ of the body and are the most striking findings in the disease (*11*).

The data to be presented include only male cases since no cases were seen or reported in female personnel with the United Nations Forces or in Korean females.

SEASONAL DISTRIBUTION

The distribution of clinically diagnosed cases in United Nations troops by week of onset is presented in Figure 1. Diagnostic criteria included fever, leucocytosis, albuminuria and hemorrhagic phenomena. Hemorrhagic fever suspects of the United Nations Forces in Korea were hospitalized in U. S. Armed Forces facilities. Because data were more available and they experienced the greatest number of cases, most of the discussion will be concerned with hemorrhagic fever in U. S. troops. While Figure 1 presents only clinical cases, subclinical cases undoubtedly occurred but because of the lack of means for identifying them no data are available on the degree of under-reporting.

The seasonal distribution as presented in Figure 1 corresponds to the findings of the Japanese (*4*) who reported seasonal peak months to be May and June and again in October and November.

The distribution of cases, during the period of this study, for major components of the United Nations Forces is given in Table 1. It will be noted that the U. S. Army experienced the largest number of cases.

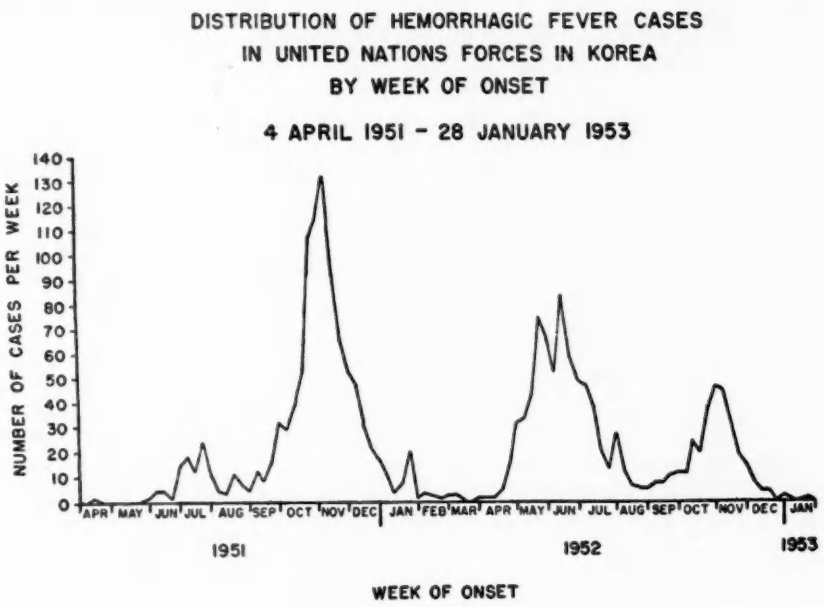

FIG. 1

Review of official reports covering the period prior to June, 1951, showed no increase in fevers of undetermined origin or diseases from which hemorrhagic fever must be differentiated. The first case in April, 1951, was diagnosed at autopsy as thrombocytopenic purpura and it was not until 17 September, 1951, upon review of the autopsy protocol and sections, that the diagnosis of hemorrhagic fever was presented. The absence of reported cases during the period July, 1950, to June, 1951, can only be speculated upon in the light of present knowledge.

The increased number of cases in the spring–summer, 1952, compared to the 1951 season is believed to be caused by deeper penetration of our forces into the endemic area. The marked decrease in the fall–winter, 1952, when compared to the same period in 1951 is attributed to the preventive measures employed by the population at risk in the known endemic area. Experience during the 1951 season showed that the incidence in a given division depended upon the sector occupied on the battle line. Arrangement of divisions in sequence according to case rates was found generally to be in the same order as in the line of battle, the high incidence appearing on the west and the low on the east end of the line. Until 1952 the divisions advanced along a south-north thrust line in their sectors. Subsequent to 1951 the divisions were shifted laterally across the front resulting in a loss of continuity in the data for a given division and a given area. Unfortunately for epidemiological purposes continuous data are available for only one division which retained its south-north thrust line over a two year period.

TABLE 1

Distribution of 2070 cases and 122 deaths and comparison of case fatalities from hemorrhagic fever by major commands for various periods from 11 April, 1951, to 28 January, 1953

PERIOD	U. S. ARMED FORCES			U. S. ARMY			U. N. FORCES			KOREAN			TOTAL CASES		
	Cases, no.	Deaths, no.	Case, fatal	Cases, no.	Deaths, no.	Case, fatal	Cases, no.	Deaths, no.	Case, fatal	Cases, no.	Deaths, no.	Case, fatal	Cases, no.	Deaths, no.	Case, fatal
11 Apr 51–30 Jan 53	1799	98	5.45	1694	92	5.43	200	13	6.50	71	11	15.49	2070	122	5.89
11 Apr 51–26 Mar 52	946	63	6.66	917	63	6.89	102	9	8.82	29	5	17.24	1077	77	7.16
27 Mar 52–28 Jan 53	853	35	4.10	777	29	3.73	98	4	4.08	42	6	14.29	993	45	4.54
11 Apr 51–15 Aug 51	89	13	14.61	86	13	15.12	Too few data for further analysis						96	16	16.67
16 Aug 51–26 Mar 52	857	50	5.83	831	50	6.02							981	61	6.22
27 Mar 52–27 Aug 52	580	29	5.00	536	25	4.66							684	35	5.12
28 Aug 52–28 Jan 53	279	6	2.19	241	4	1.66							309	10	3.24

April–August—"spring-summer season"; September–March—"fall-winter season"

GEOGRAPHIC DISTRIBUTION

The geographic distribution of 1080 cases is shown in Figure 2. The locations plotted indicate the stations of the individuals 10 to 14 days prior to the date of onset of symptoms. The main lines of resistance on 1 January, 1951, 8 July, 1951, and 31 December, 1952, have been superimposed to show that more cases appeared as the forward elements of the United Nations Forces advanced into the

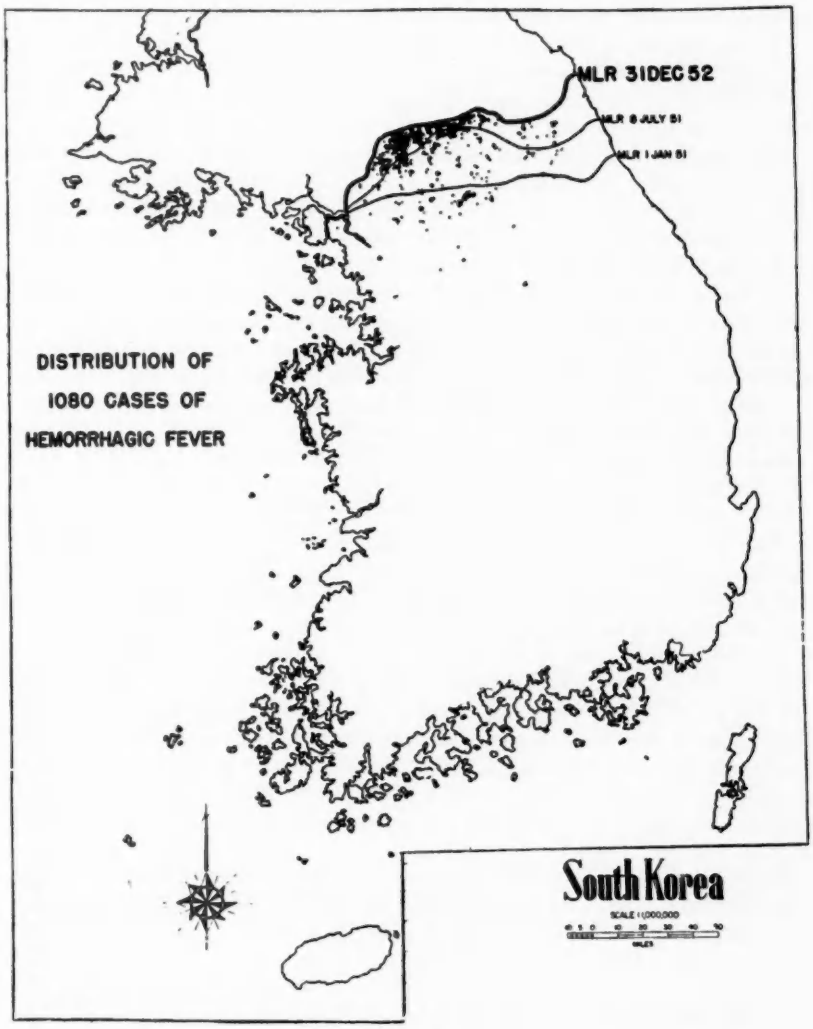

FIG. 2

endemic area. Since the Eighth Army passed over this same endemic area at least twice in the fall and winter of 1950, why were no cases of hemorrhagic fever observed or reported at that time? The answer is simple. The 1950 passages north and south through the area were made in winter when the disease incidence is normally low. Also the moves were comparatively rapid and did not permit of any prolonged stay in the endemic area. The heavy concentration of cases in the west is believed to be related to a main route of travel to and from Manchuria and the geographic ecology to be described later.

The occurrence of this disease in the native population has been a matter of interest and speculation inasmuch as none of the natives questioned had any knowledge of the disease. In view of the poor availability of medical care in Korea and the conditions existing in the country it is conceivable that the disease could remain undiagnosed. Fourteen Korean patients were questioned as to place of birth and whether they had resided at any time before the present conflict in the endemic area. Thirteen of the individuals had lived in South Korea outside of the area of known endemicity, and one was born in North Korea but had never lived in the known endemic area prior to the war.

Knowledge of the size and expanse of the endemic area is necessarily limited on the north by the main line of resistance and on the south by the actual known locations of diagnosed cases. In general the disease occurred sporadically in the endemic area south of Seoul. For the purpose of employing preventive measures the endemic area has been established as that area of Korea north of a line drawn east and west through Wonju (37°27′N) to the main line of resistance. No cases were known to have acquired the disease south of this area.

NATIONALITY AND RACE

This disease occurred in all of the United Nations units on the line except one small unit. Although the total number of cases is small in nationalities other than the British Commonwealth Division and U. S. Armed Forces, statistically their susceptibility was approximately the same as in U. S. troops.

The Koreans are susceptible to the disease but the fact that they were not cognizant of it prior to its being recognized in 1951, coupled with a system of reporting which has the language barrier as one obstacle, adds to the lack of significant data concerning the true incidence of this disease in Koreans. Evacuation of other nationalities was made through channels where records and reports were more readily accessible. It is believed that the paucity of Korean data at this time does not represent a true picture of the occurrence of the disease in the native population.

In 1535 cases for whom race was known, 87.2 per cent were Caucasian, 6.5 per cent Negroid, 5.0 per cent Mongolian and 1.2 per cent classified as of other races. Data are not available on the racial distribution in the U. N. Forces.

AGE

An age distribution of 1561 cases is presented in Table 2. The ages range from 17 to 49 years with a mean of 23.0 years and a mode of 21.8 years. Ninety per cent

TABLE 2

Distribution of 1561 cases of hemorrhagic fever by age

AGE IN YEARS	CASES NUMBER	PER CENT
17–21	504	32.29
22–26	888	56.89
27–31	95	6.08
32–36	50	3.20
37–41	19	1.22
42–46	4	0.26
47–51	1	0.06
Total	1561	100.00

Mean age—23.02 years; 90 percentile—27.36 years.

of the cases occurred in men under 27.4 years of age. It is apparent that this disease occurs mostly in those age groups most suited for combat duties in forward units. Age distributions for the population at risk were not available aud further relationship studies were not possible.

RANK

The arrangement by rank of 1694 cases in Eighth Army troops in Table 3 shows that no cases occurred among individuals above the rank of colonel during the period of study. The percentages of cases for each rank paralleled very closely the mean distribution of ranks in Eighth Army over a six months period. Although there was a distinct difference in the actual and expected incidence in private and private first class ranks it will be noted that the combined data for these two

TABLE 3

Distribution of 1694 U. S. Army cases of hemorrhagic fever by rank

RANK	CASES NUMBER	CASES PER CENT	EXPECTED* PER CENT
Pvt	230	13.6	21.7
Pfc	711	42.0	31.9
Cpl	400	23.6	23.7
Sgt	171	10.1	10.3
Sfc	72	4.3	4.2
M/Sgt	17	1.0	1.9
W O	6	0.3	0.5
Lt	47	2.8	2.9
Capt	21	1.2	1.8
Major	6	0.3	0.7
Lt Col	2	0.1	0.3
Col	1	0.1	0.1
Misc	10	0.6	0.0
Total	1694	100.0	100.0

* Percentage distribution by rank of troops in 8th Army

ranks agree very well. Since the difference in these ranks is one of pay and not of duty assignment, there is no significance attached to the variation in the data for these two groups individually. Rank in itself is not significant under the conditions which existed during the period covered by these data although it was the combat soldiers in the rifle companies in forward positions who most often contracted the disease.

BRANCH OF SERVICE

The cases from all U. S. Army divisions were grouped according to their branch of service and Table 4 shows that the infantry troops account for 63.8 per cent of the cases in the divisions which is to be compared with the 56.7 per cent expected to occur by chance in this group based on the mean strength of a division and the mean number of infantrymen in it. The difference of 7.1 per cent is statistically significant. It is obvious that this group was at greater risk to exposure. When cases for infantry and artillery personnel were combined they comprised 82.2 per cent of the total cases from divisions as compared with 70 per cent which would be expected to occur by chance in these two groups.

DEATHS

There were 122 cases which terminated in death from one to 28 days following onset, with a mean of 8.9 days and a mode of 7.2 days (Table 5). The Japanese (*4*) reported an overall case fatality rate of 15–30 with a higher mortality during the fall–winter wave than during the spring–summer wave but this was not observed in the U. S. Army cases or for the total group in this study (Table 1). The high case fatality rate in Koreans is attributable to lack of knowledge on the part of their medical officers concerning diagnosis, treatment, and pathological physi-

TABLE 4

Distribution of hemorrhagic fever cases in U. S. divisions by branch and by per cent

UNIT	CASES	PER CENT
Headquarters	29	2.1
Band	1	0.1
Military Police	5	0.4
Ordnance	10	0.7
Quartermaster	9	0.7
Replacement	2	0.2
Signal	15	1.1
Medical	15	1.1
Armor	90	6.7
Engineer	52	3.8
Infantry	857	63.8
Artillery	247	18.4
Miscellaneous	12	0.9
Total	1344	100.0

TABLE 5

Distribution of 122 deaths from hemorrhagic fever by time from onset to death in days

DAYS FROM ONSET TO DEATH	DEATHS NUMBER	PER CENT
1	4	3.3
2	1	0.8
3	3	2.5
4	8	6.6
5	13	10.6
6	10	8.2
7	20	16.4
8	11	9.0
9	12	9.8
10	11	9.0
11	4	3.3
12	3	2.5
13	3	2.5
14	3	2.5
15	2	1.6
16	2	1.6
17	4	3.3
18	3	2.5
19	—	—
20	—	—
21	2	1.6
—	—	—
25	1	0.8
26	—	—
27	1	0.8
28	1	0.8
Total	122	100.0

Mean days from onset to death—8.89 days

ology of the disease coupled with inadequate treatment facilities. Many of the Korean cases were personnel working in U. S. Army areas, diagnosed by U. S. Army medical officers, and evacuated to R.O.K.A. Hospitals.

The case fatality rate (Table 1) decreased as a result of medical officers becoming more fully acquainted with the symptoms and diagnostic criteria for the disease, early diagnosis, rapid evacuation and improvement in medical care arising from a better understanding of the disease. Also, the decrease in case fatality was closely related to the several improvements in the handling and treatment of these cases, which were instituted generally prior to the seasonal increase in incidence. As a result of the experiences gained during 1951, the Surgeon, Eighth Army, established a treatment center early in April, 1952, close to the area from which the greatest number of cases had been evacuated in 1951. A team of experienced medical officers visited each division prior to the seasonal increase to indoctrinate the unit medical officers concerning the early diagnosis and proper

handling of the suspected cases. All suspects were evacuated by helicopter to this hospital. All research activities on clinical aspects of the disease were concentrated at the center.

As in any disease, until the whole disease spectrum is known so that an accurate assay of the total number of cases experienced can be accomplished, a natural case fatality rate cannot be ascertained.

RESERVOIR

The Japanese (*4*) claimed the reservoir to be a field mouse *Apodemus agrarius*. An Eighth Army preventive medicine survey detachment trapped and shipped by air courier, during August-September, 1951, 39 lots totalling more than 200 small mammals (*11*) to the laboratory in Tokyo, including this and other local species for agent isolation studies. All Eighth Army preventive medicine units participated in trapping field rodents (*10*, *12*, *13*). Tokuda (*7*) stated that *A. agrarius* was more abundant than any other species of Muridae in Manchuria and Korea which was confirmed by our unit collections throughout South Korea wherever trapping operations were conducted. At no time during trapping or rodent control activities in the endemic area was evidence reported of an epizootic in the field rodent population.

The general consensus among officers engaged in rodent control was that the ecological conditions in the western sector of the endemic area, where most of the cases occurred, were conducive to supporting a larger rodent population than in the eastern sector. Geographically, the broader valleys in the west were more favorable for farming and rice propagation than in the east where the valleys were markedly reduced in width by the mountainous terrain which curtailed the abundance of food grains necessary to support a large rodent population. Specific rodent population data are not available but rodent poisoning was practiced more intensely in the western sectors.

VECTOR

As a result of their extensive investigations in Manchuria, the Japanese claimed the vector to be a mite, *Laelaps jettmari* Vitzthum (*4*). According to available literature on the distribution of mites in Korea (*6*), this species apparently had not been reported from Korea prior to the investigation of hemorrhagic fever although it was known in Manchuria and Japan. Few mites were recovered from the live rodents shipped to the laboratory in Tokyo. In November, 1951, live mites were collected from rodents, quick frozen in lots of 100 and shipped to the laboratory for agent isolation studies.

L. jettmari was collected on rodents throughout Korea south of the main line of resistance wherever trapping operations were conducted (*10*, *12*, *13*) and found not to be host specific. The Japanese (*4*) claim to have transmitted the agent by inoculation of suspensions of this mite collected from *A. agrarius*. In examining the index for this Laelaptid mite one is impressed by the fact that it was relatively common in the collections throughout the year and showed no correlation with the incidence of the disease.

Ticks were collected in such small numbers in the endemic area that they are not considered of importance as a vector of this disease. There were no reports of troops infested with ticks.

Flea collections from field rodents resulted in so few specimens that statistical studies on them were not warranted. Fleas presented no problems requiring control measures (except on rare occasions in urban areas) and it is highly improbable that they serve as vectors of hemorrhagic fever.

The sporadic occurrence of this disease during the winter months would appear to eliminate the mosquito as a vector.

Chiggers or larval forms of the Trombiculid mites collected by Eighth Army preventive medicine units (*10*, *12*, *13*) from field rodents showed indices which decreased to a very low level during the months of June, July, and August and increased to a high in the latter parts of April and October, about two weeks prior to the seasonal peaks of hemorrhagic fever cases. This biphasic seasonal distribution during an annual cycle, when compared with the biphasic incidence of hemorrhagic fever peaks indicates a logical and sound deduction that one of the chiggers is the most probable vector. On the other hand, mites were not recovered from any individuals presenting symptoms of the disease; there were no reactions to chiggers, such as are seen in the United States; no eschars as seen in scrub typhus have been found on patients; nor have the patients had any other indications that they had been attacked by such a vector.

INCUBATION PERIOD

No direct evidence was obtained of the incubation period; however it may be approximated by examining evidence based on the arrival and departure of individuals from the endemic area. The ideal situation for measuring the limits of incubation period in a disease occurs when the group under study is exposed to risk for one day. This did not occur in this series of 2070 cases. Therefore the best data available comprises two small groups, the first being twenty cases whose known times of arrival in the endemic area ranged from 7–36 days prior to onset, with an average time of 23.3 days. Obviously the most significant figure in this group is 7 days since this represents the minimum period of incubation. The second group of 30 cases departed the endemic area from 4–35 days prior to onset, with an average of 13.4 days. Here the significant figure is 35 days representing the maximum period of incubation. Data are not available to permit narrowing the incubation period to an average figure.

WEATHER DATA

Data on maximum and minimum temperatures and precipitation were available for Seoul City Airport, Seoul, Korea. There were no weather observations available in the presumed endemic area until the latter part of November, 1951, when weather stations were established in connection with the study of cold injuries. The observations of these weather stations were not available during the periods of maximum incidence of the disease. Korean weather data have been summarized (*8*) from available data for a number of previous years. The mean

date of first frost in the endemic area is from 10 to 20 October and the last frost in the spring from 30 March to 20 April. Frost penetration of soil averages 6 to 12 inches. The first and last frost could well explain the decrease and increase in fall and spring cases respectively. The mean annual precipitation may range from 45 to 50 inches with the heaviest precipitation during June to August when there may be 23 to 32 inches. This higher precipitation during the summer months might influence the chance of exposure to a mite vector in much the same negative way as experienced with the mite vectors of scrub typhus. The lowered incidence of the disease in mid-summer may, if the mite is proved to be the vector, be accounted for by the lessened activity of the mite in this season (*19*).

ENVIRONMENTAL FACTORS

Data have been obtained on environmental factors in 342 cases. The transmission of this disease does not appear to be associated with food: first, because of the wide distribution of cases, not only geographically, but also in relation to time of onset; second, 317 or 92.7 per cent claimed to have eaten only issued food whereas there were 25 or 7.3 per cent who admitted eating native food one or more times in the three weeks prior to onset. On similar grounds, there appears to be no reason to consider it water-borne. Treated water from multiple widespread sources was consumed by 308 or 90.0 per cent during the 3 weeks prior to onset while 34 or 10.0 per cent admitted to consuming untreated water one or more times during the period. During the warm months consideration was given to bathing in streams but there was no evidence to indicate any relationship to this disease, especially when the bulk of the cases occurred after the swimming season. In reply to an attempt to gain some idea of the existence of rodents in the areas where these individuals had been living, 251 or 73.4 per cent stated that they had seen rodents while 91 or 26.6 per cent had seen none. Insect repellent and DDT dusting powder were not employed to the extent they should have been, since 55.6 per cent of those questioned did not use repellent during the three week period prior to onset and 57.3 per cent did not use DDT dusting powder. Early in 1952 DDT dusting powder was replaced with a 1 per cent Lindane dusting powder for mass dusting and pyrethrum powder for individual application because of the resistance of Korean body lice to DDT. No study was made to determine the use of these newer formulas. The sleeping habits indicated that 219 or 63.8 per cent of those questioned had slept on the ground, in foxholes, bunkers or native huts at some time during the three week period prior to the date of onset.

This disease does not appear to be transmitted by food or water nor does bathing in native streams or rivers seem to play a part in its transmission. The few cases reported from so-called urban areas were found generally upon further investigation to have taken recent trips out of town. The disease is predominantly acquired by individuals living in rural areas.

DISCUSSION

The disease which has been described by the Japanese as epidemic hemorrhagic fever in Manchuria and the disease which constitutes the basis of this study in

Korea are identical, clinically and pathologically. Confirmation of the pathological findings were made at the 406th Medical General Laboratory on examination of pathological specimens from Japanese cases.

Japanese doctors who had seen cases in Manchuria, after examination of patients in a U.S. Army Hospital in Tokyo, also concurred in the clinical diagnosis.

Although no direct evidence has been obtained to substantiate the claims of the Japanese investigators that the agent is a virus transmitted by a mite vector, *Laelaps jettmari*, from a rodent reservoir, the mouse *Apodemus agrarius*, there has been no direct evidence to refute the claims.

Consideration must be given to the following observations: the fact that this disease is not contracted by direct contact, as evidenced by the absence of any cases in persons rendering medical care to hospitalized cases; the lack of evidence pointing to a food or water-borne source of infection; the significant findings that infantrymen are at greater risk; the double-peaked incidence which is not typical of mosquito, louse or tick-borne diseases but which is, however, found in mite-borne scrub typhus; the paucity of ticks and fleas; and the fact that one individual contracted the disease while working with rodents in an area from which cases had been evacuated and who, if a mite-rodent pair were involved, was more highly exposed to risk than the average individual who developed the disease. Although none of these observations is direct proof of the Japanese concepts, certainly they lend credence to a concept of transmission through a mite-rodent pair.

A review of the clinical and pathological descriptions in the available Russian literature on the Far Eastern nephroso-nephritis of Siberia, for the Japanese epidemic hemorrhagic fever, and findings for the disease encountered by the United Nations Forces in Korea, indicates a strong resemblance between all three diseases. Clinically and pathologically the Manchurian and Korean experiences have been correlated and it is believed that were it possible to study the Far Eastern nephroso-nephritis it might well be found to be identical, clinically and pathologically, with hemorrhagic fever.

SUMMARY

A report on the epidemiology of hemorrhagic fever based on 2070 cases and 122 deaths experienced by United Nations Forces in Korea has been presented. This disease, unknown in Korea prior to 1951, has been found to be clinically and pathologically the same disease as described by the Japanese in Manchuria.

Despite the fact that the agent has not been isolated, nor the vector or reservoir defined, available data strongly suggest that a mite-rodent pair is implicated in the transmission of the disease. The inability of other investigators to isolate the agent and develop immunological or serological procedures has limited in many respects the epidemiological study of this disease.

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HEMORRHAGIC FEVER II. PREVENTION

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The cases of hemorrhagic fever which began to appear in UN troops in Korea in April, 1951, increased and reached a peak of incidence about the middle of November, 1951 (Marshall, 1954). Epidemiological studies, preliminary studies of rodents and their ectoparasites, and review of Japanese and Russian literature on experiences with a similar disease syndrome in Manchuria, Eastern Siberia and elsewhere, pointed strongly to the probability that the disease was mite-borne from a rodent reservoir. In spite of the failure of continued laboratory research to reveal a definite etiological agent, vector or reservoir, or to accomplish animal passage of the disease, a control program against mites was planned to protect Eighth U. S. Army troops in the endemic area.

Mite control in an area roughly 120 by 150 miles in a field army where tactics and normal duties of personnel produce intimate contact with mite infested areas is a staggering problem. To reduce it to practical dimensions in the beginning, control was pursued along limited lines:

1. Individual protection by insect repellents and specially treated clothing and sleeping bags.
2. Limited area control of rodent hosts by trapping and poison baiting.
3. Spraying of tents and bunkers with 1% Lindane for mite control.

Rodent surveys in the endemic area revealed predominance of the lined-back field mouse, *Apodemus agrarius coreae* Thomas, which inhabits both grassy and wooded lands characteristic of the endemic area. These mice readily entered tents and bunkers in search of food and warmth. Control was effected primarily through use of warfarin mixed in the ratio of one part to 19 parts of cornmeal and placed in bait stations on the outside of tents and bunkers throughout unit areas. Bait stations were also placed around the perimeters of unit areas to intercept rodents attempting to enter. The stations were left permanently in place, since warfarin mixed with dry cornmeal is a fairly stable bait, and were augmented with additional bait as needed. Both warfarin and cornmeal were shipped to the theater unmixed and combined prior to use by the preventive medicine units attached to each division. This assured proper mixing and equitable distribution. Average usage was 3,000 pounds of mixed bait per division per month. Traps were used occasionally inside tents and bunkers but main reliance on control was poison bait. Success in this aspect of control was immediate and continuous throughout the Korean campaign.

The problem of personal protection of soldiers through miticiding clothing and applying insect repellents was not as easy to solve. Attempts at solution were

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frustrated and complicated by a number of factors which will be discussed briefly. The miticide used was listed by the Quartermaster as "Repellent, clothing treatment." It was composed of dimethylphthalate, dibutylphthalate, benzyl benzoate or various combinations of these compounds, with an emulsifier. The insect repellent used was the same as that used in World War II and called, "Repellent, insect, 2 ounce bottle."

The first control measures were instituted in February, 1952, in one division with the highest incidence. While dipping of outer clothing in a miticiding solution of one part miticide diluted with 17 parts of water was recognized as the best treatment, the winter weather made drying of clothing difficult so, as a test only, spraying the outer clothing every 12 days with a 1–10 miticide solution until damp was tried. Outer coverings of sleeping bags were sprayed similarly. Additionally, insect repellent was to be applied by hand every other day to boot tops, trouser bottoms, around the waist and the fly openings. Limited area control also consisted of spraying floors of tents and bunkers with 1 % Lindane emulsion.

Insect repellent was not used by troops in adequate amounts to be especially effective. It must be applied by the individual and adequacy of use depends entirely on his desire to make a thorough application. The material is oily and generally not acceptable to the average soldier. In addition, mites cannot be seen or felt like mosquitoes and the average soldier forgets to use the repellent against something of which he is not conscious.

The same philosophy applies to wearing treated clothing and soldiers were hard to convince that it was necessary. The annoying reaction of both miticide and repellent on plastic items such as fountain pens and watch crystals was another cause for non-acceptability.

The test spraying of miticide on clothing was not a successful control measure notwithstanding its advantages of speed, adaptability to winter weather while clothing was being worn, availability of handsprayers, the fact that troops usually had only one uniform in their possession, and the impossibility of organizing a dipping program quickly. The spray method was not done properly by all units. Some uniforms were sprayed correctly with an adequate treatment while others were sprayed haphazardly or not at all. Nevertheless, it was the best device immediately available so in May, 1952, the program was expanded to include all troops located north of corps rear boundaries and it became generally effective by 16 June, 1952.

Concurrently, publicity and education on hemorrhagic fever control were obtained through various media. Small pocket-sized calendar cards were printed for issue to all UN troops, with a 6-months calendar on one side and instructions for personal protection against the disease on the reverse in English, Spanish, and French, as befitted the international character of the UN Forces. Timely information was published in the Army Surgeon's Weekly Bulletin and preventive medicine units throughout the Eighth Army instructed enlisted men of each company, battery or similar unit in basic control measures and the proper application of miticide, poison baits and repellent. Short "leaders" were attached to

movie films distributed throughout the Army area, showing the proper application of insect repellent.

Another method of miticiding was tried by only one division-sized unit and illustrated further the desirability of complete dipping of clothing versus hand methods. This unit adopted a policy of applying dibutylphthalate miticide by hand to the uniforms at fortnightly intervals from 15 May, 1952, to late December, 1952. The unit remained in the same area throughout the study and experienced 69 cases from April, 1951, to April, 1952. During the next year, roughly the period when hand-miticiding was done, from April, 1952 to February, 1953, there were 74 cases. This indicated that application of miticide in this manner does not give protection comparable to clothing completely impregnated by dipping.

The alternate and more desirable method of treating clothing by complete dipping in miticide was gradually adopted after solving the problems that arose from time to time. This technic was ordered on 22 June, 1952, by a command directive which enlarged the endemic area to include all units operating North of a line drawn East and West across Korea through the City of Wonju. The directive required dipping of trousers, shirts and socks of every soldier in a 1–17 solution of the miticide twice monthly or after every third washing. The first idea was to treat all uniforms in Quartermaster field laundry units but it was learned that only about one half of the troops were serviced by these laundries. In addition, miticiding would increase laundry processing time and decrease output. Unless quotas were established, overloading of the field laundries would occur.

Laundry processed in Quartermaster field laundries could be divided into two classes, that received in exchange for clean uniforms at shower points located in army and corps areas, and the bulk clothing from line infantry regiments. The Quartermaster agreed to impregnate by dipping (Army, 1948), at field laundries, all clothing issued clean at the shower points, which amounted to approximately 4,500 uniforms per day. Bulk clothing was laundered and returned clean to the regiments where it was then impregnated by regimental personnel.

Methods for impregnation by dipping were also worked out with individual units doing their own laundry in unit areas, and with infantry line regiments for treatment of clean clothing issued to troops at regimental shower points.

The problem of clothing-impregnation in relation to the number of washings was not completely solved. In company or smaller units where laundry was done in the unit area by Korean houseboys or laundresses, the clothing was usually treated every other week. By this method, marking of items of impregnated clothing was unnecessary. In treating bulk lots of clothing at the quartermaster or regimental shower points, where thousands of garments were handled daily by Korean laborers and where the clothing exchange system meant that clothing was not necessarily returned to the same units or individuals whence it came, no practical system of marking clothing was devised. Therefore, to assure that all clothing received effective treatment, impregnation was done before each issue or after every washing. This method required more miticide than was necessary

to provide adequate protection but was judged necessary in view of the difficulty of separating and giving special handling to clothing in order to provide retreatment after certain periods of wear or a certain number of washings.

Experience in 1951 showed a definite increase in hemorrhagic fever incidence beginning in September and reaching a peak in November. It appeared that the maximum number of infections were acquired during late October at the time of change from summer to winter clothing. Therefore, logic and economy directed the impregnation of winter clothing prior to issue to troops. It would have been desirable to do this prior to bulk issue of clothing by Quartermaster to units but this was impractical because of labor requirements and lack of baling material to rebale the clothing after treatment. As a less-desirable alternative, on 7 July, 1952, a directive was published requiring all units to dip winter trousers, shirts and socks in 1–17 miticide solution before issuing them to troops.

To provide protection for replacements arriving in Korea until they could reach their initial unit and have their clothing impregnated, a request was made to the Far East Command on 14 July, 1952, that replacements be issued impregnated clothing before leaving Japan. This request was approved on 18 September, 1952, and actual issue of impregnated clothing to Korea replacements began on 15 October, 1952.

By 22 September, 1952, the control measures for combined attack on hemorrhagic fever included dipping of clothing in 1–17 mixture of miticide and water twice monthly or after every third washing, requiring all winter clothing to be treated before issue to troops, spraying the outer cover of sleeping bags with a 1–10 solution of miticide, use of insect repellent by individuals, spraying of tent and bunker floors and walls with a 1% Lindane emulsion every two weeks, and continuous rodent control by warfarin poison baits. These measures are still in effect.

In April, 1953, impregnation of bulk laundry at the quartermaster field laundries was improved by establishing laundry quotas and substituting miticide solution for the third rinse. Subsequent operations indicated that the recommended dosage of 7 pints of miticide to 3½ gallons of water per washer load, although sufficient to repel ticks, was more than the amount needed to produce a safe knockdown for mites. The dosage was therefore reduced to 4 pints of impregnant (45% benzyl benzoate and 45% dibutylphthalate) to 3½ gallons of water per washer load, added through the soap powder slot of the machine.

RESULTS OF CONTROL MEASURES

The evaluation of control measures against hemorrhagic fever is difficult because of limited information on its seasonal incidence in an unprotected population. The cases in June and July, 1951, occurred at the end of the spring-summer period of increased incidence and the number of troops at risk was not as large as later. The fall-winter 1951 period of increased incidence is believed to represent a true experience with the disease inasmuch as no preventive measures had been instituted at that time. The spring-summer 1952 experience showed a significant rise but, since miticide spray of clothing had been started and data from previous

seasons was lacking, it is not known if any appreciable number of cases was prevented.

The first comparative data available cover the period 29 August, 1952, to 30 January, 1953, compared with the same period one year earlier. This comparison shows that for a known standard population in the Eighth U. S. Army exposed to risk there were 195 cases compared to 705 cases the year before. This means 72 per cent fewer cases occurred during the period when impregnated clothing was being worn than during the period when no control measures were in effect. Clothing impregnation was never complete throughout the Eighth U. S. Army but the percentage of completeness was estimated to be greater than 72 per cent.

Epidemiologically it would be desirable and rewarding to compare incidence in the two fall-winter periods of 1951–52 and 1952–53 by units, say divisions, but this is not possible since tactical movements shifted the divisions from one part of the endemic area to another so that the exposure to risk was not the same. In addition, some divisions pursued the control program with more vigor than others. The preventive program was best served and supported wholeheartedly where a case or a death occurred in the unit.

However it is possible to illustrate the effect of impregnation of clothing in one company, and this is presented graphically in Figure 1. This unit went from line to reserve status late in June, 1952. A program of physical training was started in the unit about 1 July in which the men exercised in shorts, socks and boots, doing calisthenics and a mile run daily at 5:30 AM. Frequently push-ups were included in the exercises. In a period of 27 days, beginning 20 days after the calisthenic program was started, 14 cases of hemorrhagic fever occurred in this unit. Clothing had been impregnated three times during the period 20 July to 18 August. After 18 August, as a result of investigation by the division preventive medicine officer, calisthenics were conducted in impregnated clothing. From that

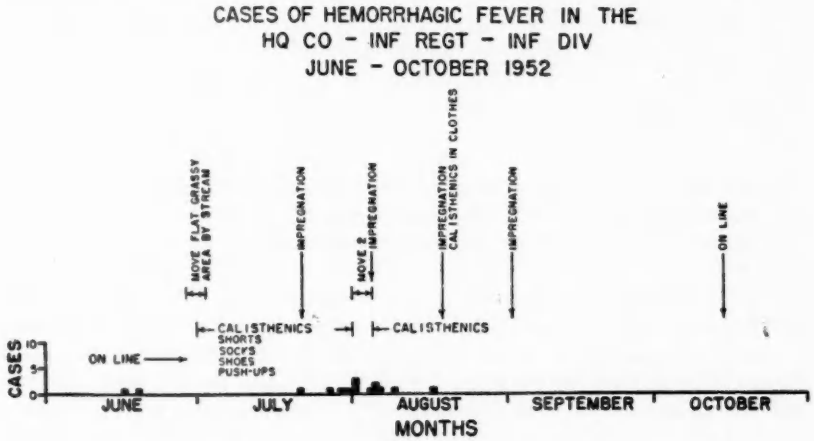

FIG. 1

time until the unit returned to the line on 15 October there were no more cases. This experience indicates that wearing of impregnated clothing afforded individual protection against the disease.

DISCUSSION

The measurement of the effect of preventive measures against a disease presents many problems. In the case of hemorrhagic fever the inability positively to establish the mite as a vector or to prove the definitive host reservoir, coupled with the movements of the troops at risk and the lack of sufficient data on seasonal occurrence over a long period of time prior to the inauguration of an intensive preventive program, complicates the problem of evaluating preventive measures.

The reduction of 72 per cent in the number of cases experienced in a known standard population in the Eighth U. S. Army in Korea during the period 29 August, 1952, to 30 January, 1953, when compared with the same period one year earlier is significant. The area in which troops maneuvered remained much the same, with slight gains, from September, 1951, to the present. Because of military operations it was not possible to establish a controlled study in this population, either with respect to preventive measures or seasonal occurrence.

It is believed that the hand application of miticide to outer clothing does not afford protection in view of the experience of one division during the period of study. Also, spray application of miticide to outer clothing was found to be unsatisfactory because of irregular application and inadequate wetting. It is questionable if the application of miticides by these two methods could furnish adequate protection in a mass program. If properly used by a few experienced individuals, who know how to apply the miticide and have a desire to do it properly, it might be of value when dipping is impossible.

Since the 72 per cent reduction in cases occurred in a population where a high percentage (probably more than 72 per cent) were wearing dip-impregnated clothing, it is believed that for a mass program such as in a field army where combat troops wear their clothing almost 24 hours per day, this will furnish the highest degree of protection and is the method of choice.

SUMMARY

Personal protection is the most important single aspect of the control program against hemorrhagic fever and consists of personally applied insect repellent and impregnating the outer clothing by dipping it into a standard miticide, 1–17 dilution, at least once monthly or after every third washing, preferably at the time of laundering. Bulk laundry from line regiments and clothing from army shower points should be impregnated at quartermaster field laundries. When this service is not available to troops, impregnating should be done locally by hand dipping. It is obvious that the fewer the impregnating stations the more effective will be the program. The other phases of control—spraying outer covers of sleeping bags with 1–10 miticide, spraying 1% Lindane emulsion to tent and bunker

floors and walls, and the rodent control program—all add to the completeness of the program.

Control of mites at best is difficult and, during military operations, personal protection against them plus limited environmental control against both mites and possible host reservoirs make a sound approach to reducing the number of non-effectives from this disease to a minimum.

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TREATMENT OF SCRUB TYPHUS IN THE PESCADORES ISLANDS WITH CHLORAMPHENICOL, AUREOMYCIN AND TERRAMYCIN[1]

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An epidemic of scrub typhus appeared among soldiers of the Nationalist Chinese armies in the Pescadores Islands in the summer of 1952. These islands, sixty-four in number, are located in the South China Sea between Taiwan and the mainland of China. In 1931, Yamamiya (1935) recognized the first case of the disease there, and these islands have since been known as a hyperendemic focus of the disease (Kawamura and Yamamiya, 1935; Morishita, 1942). The recent migration of numbers of Nationalist Chinese troops to these islands introduced a large, presumably nonimmune population.

In 1948, Smadel and the Army Medical School Research Group established the value of chloramphenicol in the treatment of the disease caused by the Malayan strain of *Rickettsia orientalis*. Later aureomycin and terramycin were also found valuable against the Malayan strain by the same group (Bailey *et al.*, 1951). In view of the heterogenicity of *Rickettsia orientalis* in different locales, it was desirable to determine whether these drugs had an effectiveness against the Pescadores strains comparable to that found against the Malayan strains (Smadel, 1952). When the first cases of scrub typhus began to appear among Nationalist Chinese soldiers stationed in these islands, plans were made by members of the Nationalist Chinese National Defense Medical Center and members of Fleet Epidemic Disease Control Unit Two for conducting these therapeutic studies and concurrent epidemiologic investigations.

This report is concerned with an evaluation of chloramphenicol, aureomycin, and terramycin in the treatment of 47 cases of scrub typhus acquired in the Pescadores Islands under natural conditions of exposure.

MATERIALS AND METHODS

General. Through the cooperation of the Commander of the Nationalist Chinese ground forces in the Pescadores, medical units of the military establishment were alerted to refer all cases of suspected scrub typhus to a central hospital facility in the city of Makung. Upon admission, a medical history was obtained and a physical examination was performed. Routine blood and urine studies followed and blood was collected for rickettsial isolation and Weil-Felix studies.

[1] The opinions expressed herein are those of the authors and cannot be construed as reflecting those of their governments.

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Selection of cases. All cases were members of the Nationalist Chinese armed forces referred to us by physicians of the Nationalist Chinese Medical Corps. The cases came from endemic areas of scrub typhus and were selected on the basis of having the pathognomonic eschar, and symptoms and signs compatible with the disease. All had an acute febrile illness with a majority of these features: toxemia, headache, chills, conjunctival injection, generalized lymphadenopathy and a generalized skin eruption. Of the 50 cases of the disease, 48 exhibited the eschar. A verification of the clinical diagnosis was sought by demonstrating rickettsemia and a rise in OX-K titer in convalescent sera whenever laboratory facilities permitted. A few cases of doubtful etiology were eliminated from the study group.

Of the two patients without eschars, one demonstrated a rickettsemia and an OX-K titer of 1:1280 on the fourteenth and thirty-fourth days after onset of illness. The other case had an acute febrile illness with generalized lymphadenopathy, an acute impairment of hearing and an OX-K titer of 1:160 on serum drawn on the twenty-second day of illness.

Rickettsial isolation. Rickettsemia was demonstrated in 9 out of 18 cases. Venous blood was clotted, the serum separated, and the clot ground in a Ten Broeck grinder using buffered (Sorensen's phosphate buffer) isotonic saline of pH 7.4 as diluent to make a 10 per cent suspension. The suspension was centrifuged at 3,000 r.p.m. for 10 minutes. The supernate was separated and inoculated in 0.5 ml. aliquots intraperitoneally into five 12 to 14 gram Swiss albino mice. These were then observed for the appearance of ascites. Repassage was not attempted prior to 14 days post-injection, unless the animal died or showed ascites. Dead or chloroformed animals were necropsied; smears of the splenic surface were made, some fixed by heat and others by methyl alcohol, and stained by standard Giemsa, Machiavello and Gram technics. The presence or absence of rickettsia-like bodies in enlarged mesothelial cells was then noted. Repassage tissues consisted of a 10 per cent suspension of ground spleen, liver and/or brain in buffered isotonic saline which contained 1,000 units of crystalline penicillin G per ml. of diluent. After centrifugation, 0.5 ml. of the supernate was inoculated intraperitoneally into Swiss mice. Ascites was discernible in about 30 per cent of the animals after 14 days. By the third passage, ascites was regularly produced in 90 per cent of the animals which contained 3–5 ml. of a clear or hemorrhagic viscous fluid in the abdomen. Splenomegaly and hepatomegaly were universal, while about 50 per cent had enlarged and hyperemic subcutaneous lymph nodes. A few had a patchy pneumonitis and pleural effusion.

Weil-Felix studies. Acute and convalescent sera were routinely tested against standardized suspensions of OX-K, OX-2, and OX-19 strains of Proteus organisms employing technics currently in use at the Army Medical Research and Graduate School.

OX-K titers were performed on all but three cases. Of these three, one had rickettsemia and an eschar; the other two had eschars and a characteristic clinical picture. OX-K titers were elevated above 1:20 in all sera tested save case 5 which failed to demonstrate a rickettsemia or an OX-K rise although it was clinically a typical case of scrub typhus.

Routine clinical laboratory procedures. Erythrocyte, leukocyte, differential

white blood cell counts and urinalyses were performed according to methods employed at the U. S. Naval Medical School.

TREATMENT

Forty-seven cases of the disease were treated. Treatment was confined to the use of chloramphenicol, aureomycin, and terramycin. These were obtained as 0.25 gram capsules from standard Navy supply stock.

Smadel *et al.* (1949), on the basis of experiences with chloramphenicol, emphasized the need of avoiding a suppression of rickettsial growth too early for the immune forces of the host to have had an adequate opportunity to respond to the rickettsial agent. Smadel's studies (1949) evolved the use of a 5.0 to 6.0 gram total dose schedule given within 24 hours.

Guided by the concepts and lessons derived from the preceding studies, it was determined that none of the cases would be treated unless at least five days of clinical disease had been experienced, save where the seriousness of the patient's condition demanded immediate treatment. In addition, a closely similar plan of therapy was adopted. Initial treatment was attempted with a single dose of 6.0 grams of the drug. However, vomiting was induced and the treatment was modified to two doses of 3.0 grams each given 12 hours apart. Later, when this schedule was found successful, a regimen employing a 3.0 gram initial dose followed by a 2.0 gram dose 12 hours later was used.

Three cases received no treatment. Cases 11 and 20 received no treatment because their illness was in a late and waning stage—in the forty-fourth and thirtieth days following inception. Case 37 was mildly ill and no treatment appeared to be indicated.

The plan to treat the patients in strict rotation was only in part realized due to limitations imposed by a shortage of aureomycin and terramycin at the outset of the study. Thus the bulk of cases received treatment with chloramphenicol. The criterion of treatment evaluation was the time that it took for the temperature to return to normal. Kawamura and Yamamiya (1939), observing 32 cases of the disease in these islands, noted that fever was present in the usual case for from 22 to 37 days, or even longer. In Smadel's Malayan series (1949), the untreated control series averaged 14 days of fever.

RESULTS

Fifty cases of the disease were seen. In all, 47 cases were treated, twenty-five with chloramphenicol, 12 with aureomycin, and 10 with terramycin. The average case in the chloramphenicol and aureomycin series was treated on the ninth day of illness, but in the terramycin series, on the seventh day of illness. Clinically, all three drugs were found effective. Clinical, laboratory and therapeutic data on these three series are summarized in Table 1. No fatalities occurred in this group while previously, in untreated cases of the disease in these islands, the mortality averaged seven per cent (Morishita, 1942).

The chloramphenicol-treated cases had an average duration of fever of 40 hours after treatment was instituted. Clinical improvement was prompt in the majority of cases. Some patients felt distinctly improved within 24 hours, at times

TABLE 1

Comparison of scrub typhus cases treated with chloramphenicol, aureomycin, terramycin Pescadores Islands, Taiwan, 1952

	CHLORAMPHENICOL TREATED	AUREOMYCIN TREATED	TERRAMYCIN TREATED
Number of cases	25	12	10
Ave. day of illness Rx begun	9.5	9.0	7.4
Ave. total dose, first attack (gms)	5.2	5.5	5.85
Ave. dur. of fever (hrs)	40	48	49
Relapses	5 (20%)	2 (17%)	2 (20%)
Ave. total dose in Rx of relapse (gms)	3.0	3.5	4.5
Ave. days of hosp.	15	20	21

before the temperature had returned to normal. Ten cases (40 per cent) had fever that lasted over 40 hours—up to 104 hours—after the initiation of treatment.

Three of the cases which did not appear to be too seriously ill were given lower doses of the drug. Case 1, a 24 year old male, was admitted with a history of seven days of fever, headache, anorexia, weakness and joint pains. He had an eschar in the right lower quadrant of his abdomen and a generalized maculo-papular eruption. His admission temperature was 39°C. The patient was given 3.0 grams of chloramphenicol over a period of 12 hours with a return of his temperature to normal in 48 hours. Five days after the institution of treatment his temperature rose several degrees. An additional 3.0 grams of drug brought the temperature back to normal in 44 hours. Case 8, a 21 year old male, was moderately ill for four days with signs and symptoms similar to Case 1. He demonstrated a rickettsemia just prior to treatment. The admission temperature was 37.8°C. He received 3.0 grams of chloramphenicol and was afebrile in 20 hours. Case 32, a 23 year old male, was admitted after an illness of 12 days. He complained of chills, fever, headache, anorexia and joint pains, and exhibited a chest wall eschar, generalized lymphadenopathy and conjunctival congestion. His temperature was 38.7°C. A single dose of 1.5 grams of chloramphenicol was administered and he became afebrile in 36 hours. The favorable response in this case to such low dosage may have been influenced, at least in part, by the 12-days' duration of his illness.

The aureomycin treated group responded similarly, with temperatures returning to normal, on the average, 48 hours after the institution of treatment. Four (30 per cent) of the twelve cases continued to have fever more than 48 hours—76, 88, 92 and 92 hours respectively.

The terramycin treated cases appeared to respond less favorably clinically, although defervescence occurred, on the average, 49 hours after the onset of treatment much as in the aureomycin treated group. Four (40 per cent) of the ten cases continued to have fever 49 hours after treatment was begun—64, 76, 92, and 120 hours respectively.

The average chloramphenicol treated case was hospitalized 15 days, while the aureomycin and terramycin treated groups averaged 20 and 21 days respectively.

TABLE 2

Relapses in scrub typhus, Pescadores Islands, Taiwan, 1952

CASE NO.	DAYS OF ILLNESS	INITIAL Rx (GMS)	DUR. OF FEVER	DAY OF RELAPSE	RE-Rx (GMS)	DUR. OF FEVER
Chloramphenicol treated cases						
1	7	3.0	48 hr.	5	3.0	44 hr.
2	7	5.0	22	6	5.0	16
3	20	5.0	16	6	4.0	12
46	6	5.0	88	7	1.0	64
50	4	5.0	28	6	2.0	44
Aureomycin treated cases						
28	5	5.0	32	5	4.0	16
38	5	5.0	88	4	3.0	116
Terramycin treated cases						
26	4	5.0	24	4	4.0	52
35	2	5.0	76	5	5.0	72

RELAPSES

Recurrence of fever, usually though not necessarily associated with aggravation of symptoms, was defined as a relapse. The febrile rise was distinct, at least one or two degrees. The appearance of a relapse occurred on the average on the fifth day after initiating treatment. In general, the relapses were treated differently using smaller doses, 0.25 or 0.50 gram every six hours, until a favorable response was obtained. Table 2 presents data on the relapses.

In the chloramphenicol series, five cases (20 per cent) relapsed. The average case received 3.0 grams of drug for the relapse. A febrile remission occurred 34 hours on the average after treatment was begun. Save for Case 3, ill for 20 days, the duration of illness prior to initial treatment of this group ranged from 4 to 7 days.

Two cases in the aureomycin series and two in the terramycin series relapsed. The duration of illness was no greater than five days in any of these cases before initial treatment was instituted. In the aureomycin series, Case 28 received 4 grams of drug and became afebrile in 16 hours, while Case 38 received 3.0 grams and remained febrile for 116 hours. In the terramycin series, Case 26 was given 4.0 grams of the drug and became afebrile in 52 hours, while Case 35, after receiving 5.0 grams of terramycin, remained febrile for 72 hours.

DISCUSSION

Naturally acquired scrub typhus due to strain(s) found in the Pescadores Islands can be treated successfully with chloramphenicol, aureomycin and terramycin. Our results, using drugs, doses and schedules similar to those employed by Bailey *et al.* (1952) in Malaya, are closely comparable—Table 3. In the majority of the cases, as in Bailey's subjects, defervescence was complete within two

TABLE 3

Comparison of treatment of Malayan with pescadores strains of scrub typhus

TREATMENT	MALAYAN STRAIN*		PESCADORES STRAIN	
	No. of cases	Duration of fever	No. of cases	Duration of fever
Chloramphenicol	8	35.4 hr.	25	40 hr.
Aureomycin	5	28.0	12	48
Terramycin	8	46.9	10	49

* Bailey et al. in antibiotics and chemotherapy **1:** 16–30, 1951.

days following doses of 5.0 to 6.0 grams of drug given orally in two doses over a 24 hour period. Smadel *et al.* (1952) felt that a 5.0 gram dose over a 24 hour period was generally adequate, but that in the more seriously ill, treatment should be continued for an additional day or two.

In an earlier study, Smadel *et al.* (1949) treated 69 cases with chloramphenicol, with the majority of cases receiving 6.0 grams of drug orally within 24 hours. On the average, these cases were afebrile in 32 hours irrespective of the time during the course of the disease when treatment was begun. In our series, those treated before the ninth day of illness, which represents the mean day that treatment was begun, became afebrile on the average in 52 hours, while those treated after the ninth day became afebrile in 32 hours. It is possible that the development of immunity in the later stages of the disease contributed to a more rapid defervescence.

In our cases, as in the Malayan cases, a marked improvement in the sense of well-being frequently paralleled the drop in temperature. However, some of the patients continued to complain of weakness for one or two weeks and even longer. Most patients were ready to return to duty at the end of the third week after the onset of the illness.

Smadel (1952) stated that relapses are practically never seen if treatment is begun on the seventh day or later. "Such recurrent episodes have not been observed in patients who recovered without benefit of nonspecific therapy" (Smadel, Bailey and Diercks, 1950). These same investigators found that recrudescence occurred in one out of two cases started on treatment in the second or third days of illness. They interpreted this situation as a result of the loss of the rickettsiostatic effect of the drug prior to the development of adequate immunity to control the growth of *Rickettsia tsutsugamushi*. Our studies tend to bear this out (cf. Table 2) as six out of the nine relapses received initial treatment between the second and sixth days of illness. Two received treatment on the seventh day and one on the twentieth day of illness. In the latter group, one received an initial dose of 3.0 grams, the remainder having received 5.0 grams in initial therapy. It appears therefore that the relatively shorter duration of illness prior to the beginning of treatment is an important factor in the causation of relapses.

Smadel *et al.* (1950) recommend control of the relapse with a supplementary course of 3.0 to 4.0 grams of antibiotic. All cases in our series receiving 4.0 to 5.0 grams responded satisfactorily. It is worthy of note that 1.0 gram of chloram-

phenicol brought the temperature down to normal in 64 hours in case 46, while 2.0 grams effected defervescence in 44 hours in case 50. However, Case 38, treated with 3.0 grams of aureomycin for the relapse, failed to respond satisfactorily, with fever continuing for 116 hours.

A valid comparison of the relative efficacy of these drugs, either in the initial treatment or in the treatment of the relapse, cannot be made from our studies.

No adverse effects of the drugs, save for transient nausea and emesis, were noted, though the patients were not observed for a sufficiently long period to determine any disturbance of hematopoietic function.

SUMMARY

Treatment of naturally acquired scrub typhus due to the Pescadores strains of *Rickettsia orientalis* can be successfully accomplished with chloramphenicol, aureomycin and terramycin. A total dose of 5.0 to 6.0 grams of any of these drugs administered orally in two doses within 12 to 24 hours will control the disease in the majority of the cases. Relapses tend to be more frequent in those treated early, but are controlled with doses of 4.0 to 5.0 grams of drug. The Malayan and Pescadores strains of *Rickettsia orientalis* are closely similar in their clinical response to these antibiotics.

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AN AMEBIASIS SURVEY IN A VETERANS ADMINISTRATION HOSPITAL, CHAMBLEE, GEORGIA, WITH COMPARISON OF TECHNICS

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In response to a request of the Veterans Administration, the Communicable Disease Center of the U. S. Public Health Service conducted a stool survey for *Endamoeba histolytica* at the Lawson VA Hospital, Chamblee, Georgia, in June, 1951. Results of this study were to be used for comparison with data obtained in other VA installations. Plans for conducting the survey were developed with the approval of the VA Tropical Medicine Consultants. An attempt was made to insure that the procedures were at least as sensitive as those used in other VA hospitals and in surveys of World War II veterans.

Although it was originally planned to examine over 500 World War II veterans, the survey was terminated in April 1952 after examination of 400 patients because of the moving of the hospital.

METHODS

Selection of patients. In general, the individuals examined in this survey were ambulatory patients from the medical wards of the hospital. Patients with diabetes, peptic ulcer, infectious hepatitis, and epilepsy were excluded from the survey. Only four of the patients were females and 26 were colored. Each patient examined was given 30–45 ml. of saturated solution of magnesium sulfate at approximately 7:45 a.m. Usually this was after the patients had eaten breakfast. The patients were directed to assemble at 8:30 a.m. in an empty ward which had been established as a laboratory.

Collection of specimens. In the examination of the first 300 patients, an attempt was made to collect three post-cathartic specimens from each patient. However, since an analysis of the results obtained with 167 patients submitting three specimens each demonstrated that the third specimen was not contributing significantly to the results, only two specimens were collected from the last 100 patients in the survey (Table 1). A total of 1,034 specimens was collected from the 400 individuals examined—an average of 2.6 specimens per patient. A portion of one of the specimens from each patient (usually the last collected) was preserved in PVA-fixative (Brooke and Goldman, 1949).

Examination technics. Each specimen collected was examined immediately by direct wet mounts employing saline, iodine, and Quensel's solutions. The direct examinations were performed in the ward where the specimens were collected. After all of the direct examinations had been performed, the specimens were taken to the parasitology laboratories of the Communicable Disease Center,

[1] Lawson Veterans Administration Hospital, Veterans Administration, Chamblee, Georgia. (Now Atlanta Veterans Administration Hospital, Atlanta, Georgia)

TABLE 1

Results of direct examinations of 167 patients submitting 3 specimens each (all species of amebae included)

SPECIMENS	PATIENTS POSITIVE FOR AMEBAE		AMEBIC INFECTIONS NO.	CUMULATIVE TOTAL	
	No.	%		Positive patients No.	Amebic infections No.
1st Spec.	23	15.0	31	23	31
2nd Spec.	26	15.6	31	26	34
3rd Spec.	22	13.2	26	26	34

where the subsequent examinations were made. One specimen from each patient (generally the first) was concentrated by the Ritchie (1948) formalin-ether sedimentation procedure. Two films were prepared from each specimen preserved in PVA-fixative and were allowed to dry thoroughly. Routinely the films were stained by the Tompkins-Miller (1947) phosphotungstic acid procedure. All specimens containing questionable organisms or *E. histolytica* trophozoites or cysts were also stained by the more critical Heidenhain hematoxylin technic.

Although cultivation was not selected as one of the survey techniques, one specimen (generally the second or third) from each of 374 patients was cultured on modified Boeck and Drbohlav's medium as part of another study. The details of this collateral investigation will be reported later, but the additional organisms found by cultivation are included in the following results of the survey.

RESULTS

Parasites found. Table 2 presents the prevalence of the parasites found in the 400 patients examined. The figures represent the combined results of 1,034 direct examinations, 400 Ritchie formalin ether concentrations, 400 hematoxylin stains of PVA films, and 374 cultivations. No parasites were found in 63.8 per cent of the patients. In the remaining 145 patients a total of 218 parasitic infections was found. Thirty-seven patients, or 9.3 per cent were infected with *E. histolytica*. All of the *E. histolytica* infections were confirmed in stained smears prepared from the stool specimens or cultures preserved in PVA-fixative.[2] Twenty-four of the thirty-seven infections were confirmed by finding the cystic stage; the remaining 13 were diagnosed on the basis of trophozoites only. Twenty-five of the individuals harbored *E. histolytica* organisms (trophozoites or cysts) that measured less than 10 microns in diameter. Nine had organisms larger than 10 microns, and 3 apparently had organisms of both sizes.

Comparison of technics. Cultivation was responsible for the addition of 11 "positive patients" and 24 identified amebic infections (1 *E. histolytica*, 2 *Endamoeba coli*, 12 *Endolimax nana*, 3 *Iodamoeba bütschlii*, and 6 *Dientamoeba fragilis*). These additions resulting from cultivation are included in the results of the survey (Table 2), but are omitted from Tables 3 and 4 since cultivation was

[2] A set of reference slides has been established with the mechanical stage graduations recorded on each slide for representative specimens of the *E. histolytica*.

TABLE 2

Parasitological results of examination of post-cathartic stool specimens collected from 400 ambulatory patients at Lawson VA Hospital

PARASITOLOGICAL RESULTS	NUMBERS	PERCENTAGES
Patients with no parasites found	255	63.8
Positive patients	145	36.2
Patients with one infection	87	21.8
Patients with two infections	42	10.5
Patients with three or more infections	16	4.0
Patients positive for amebae	121	30.3
Amebae found:		
Endamoeba histolytica	37	9.3
Endamoeba coli	45	11.3
Endolimax nana	81	20.3
Iodamoeba bütschlii	9	2.3
Dientamoeba fragilis	13	3.3
Questionable or unidentified amebae	10	2.5
Other organisms found:		
Giardia lamblia	14	3.5
Trichomonas hominis	5	1.3
Hookworm-eggs	7	1.7
Strongyloides stercoralis larvae	3	.8
Trichuris trichiura eggs	2	.5
Ascaris lumbricoides eggs	1	.3
Enterobius vermicularis eggs	1	.3

not a primary technic established for the survey and will be the subject of a subsequent paper. Nevertheless, it is well to mention in this paper the results of cultivation relative to *E. histolytica*. Specimens from 36 of the 37 patients found to harbor *E. histolytica* were subjected to cultivation. Although cultivation revealed one of the infections of this species which was not discovered by the other procedures and assisted with the confirmation of five others, it failed to diagnose six of the *E. histolytica* infections. In three instances organisms failed to grow and in three the organisms which were present in the cultures could not be identified. Five of the six were infections of the small race.

Table 3 presents a comparison of the amebic infections found in unpreserved specimens with those found by examination of the portions preserved in PVA-fixative. In 110 individuals found to harbor amebae, 73 per cent of the positives were demonstrated by the direct and Ritchie sedimentation technics and 97 per cent by the PVA-fixative technic. Likewise, of 161 amebic infections identified, 64 per cent were demonstrated by the technics used on the unpreserved portions and 92 per cent by technics used on the portions preserved in PVA-fixative. This advantage of the PVA-fixative technic is due in part to the fact that post-cathartic specimens were examined. In such specimens trophozoites are likely to predominate, and apparently did in this study on the basis of the total trophozoites and cysts recorded in Table 4. Although other factors (such as pressure of time, reliance on subsequent examinations, and perhaps the adverse effect of a

TABLE 3

Comparison of amebic infections found by direct and concentration technics (unpreserved specimens) and by stained smears from specimens preserved in PVA-fixative.

TECHNICS	PATIENTS POSITIVE FOR AMEBAE		AMEBIC INFECTIONS	
	No.	% of total	No.	% of total
Direct and concentration	80	73	103	64
Stained smears (PVA-fixative)	107	97	148	92
Totals by three techniques	110[1]		161[2]	

[1] Excludes 11 additional patients positive only on cultivation.
[2] Excludes 24 additional amebic infections found only on cultivation.

cathartic on the organisms) may have had an effect, it is interesting that the PVA-fixative technic revealed over twice as many of the trophozoite infections even though the direct examinations were performed on over twice as many specimens. Although the PVA-fixative technic was more effective for trophozoites, Table 4 demonstrates the advantages of the direct and the Ritchie technics in the identification of cystic amebae. These observations confirm the desirability of employing multiple technics to facilitate the recovery of both stages of the intestinal amebae (Goldman and Brooke, 1953).

Symptomatology of patients infected with E. histolytica. Each of the case histories of the 37 patients infected with *E. histolytica* was carefully studied for evidence of symptomatic amebiasis. Special attention was directed to the current symptoms and physical signs, and to history of diarrhea or dysentery. Although 23 of the patients received amebicidal therapy after the present infection was discovered, the resulting information is of little value since the post-treatment observations were inadequate in most instances.

Four of the patients were considered to have current symptoms due to the presence of *E. histolytica* (10.8 per cent of the infected patients). The clinical diagnoses for the four cases on admission were possible amebiasis, undiagnosed dysentery, undiagnosed diarrhea, and anxiety state. All four had a history of

TABLE 4

Amebic trophozoites and cysts found by direct and concentration technics and by stained smears from specimens in PVA-fixative

TECHNICS	AMEBIC TROPHOZOITE INFECTIONS[1]		AMEBIC CYST INFECTIONS[1]	
	No.	% of total	No.	% of total
Direct and concentration	54	37	95	98
Stained smears (PVA-fixative)	135	93	51	53
Totals by three techniques	145		97	

[1] Includes mixed infections of trophozoites and cysts.

diarrhea, one dating back to military service in Greenland in 1943 and another to service in the Philippines in 1945. Three of the four patients with clinical amebiasis were infected with large race *E. histolytica*.

In five other patients, although it was considered that other conditions were responsible for their current complaints, the presence of the *E. histolytica* infection may have contributed to the symptomatology (13.5 per cent of the infected patients). The principal diagnoses were a duodenal ulcer, possible gall bladder trouble, possible ulcerative colitis, anxiety state, and strongyloidiasis. With the possible exception of the last, these patients did not have recent histories of diarrhea. Three reported having had diarrhea while in service in the United States and in Europe. Four of the five harbored small race *E. histolytica*. The patient with strongyloidiasis had large forms of the ameba.

Twenty-eight of the infected patients (75.7 per cent) were considered to have no evidence of symptomatic amebiasis. None of these patients had a recent history of diarrhea. Available information on 26 of the patients indicated that seven of them had diarrhea while in military service. Twenty of these patients harbored small forms of *E. histolytica*, three had large, and five both small and large forms.

Personal and environmental data in relation to infection. At the time of collection of specimens, a questionnaire was filled out for the patients relative to the patients' residence in rural or urban area home, toilet facilities, income, occupation, history of diarrhea, dysentery, hepatitis, and jaundice during or after military service, length and location of military service, military combat, and personal data such as age, sex, and color. The information on military combat and the location of overseas service is not considered to be reliable and will not be discussed.

Tabulations of the remaining pertinent data were prepared for the 37 patients infected with *E. histolytica* and for the 363 patients in whom this organism was not found. Statistical comparison of the two tables revealed no significant differences except in regard to toilet facilities. Twenty-two per cent of the negative patients and 55 per cent of the *histolytica* patients reported having outside toilets (P less than .01). The infected group also had higher percentages of low incomes (less than $2,000), comprised a younger age group (20 to 29 years), and had urban residence, but the differences are not statistically significant.

SUMMARY

1. Four hundred ambulatory patients in the medical wards of Lawson Veterans Administration Hospital were examined for the presence of *Endamoeba histolytica* and other intestinal parasites. An average of 2.6 post-cathartic specimens per patient was examined by direct wet mounts, the Ritchie formalin-ether sedimentation procedure, and the PVA-fixative technic. Cultivation was used on specimens from 374 of the patients. Thirty-seven (9.3 per cent) patients were found to be infected with *E. histolytica*. This rate is within the usually accepted range for the incidence of *E. histolytica* in the general population of the United States (Craig and Faust, 1951). Four of the 37 (or 10.8 per cent) were

considered to have clinical amebiasis. Despite the lapse of several years after the end of World War II, two of the four amebiasis cases could conceivably have been service-connected.

2. Comparison of the technics performed demonstrated the desirability of examining permanently-stained smears for amebic infections. The PVA-fixative technic provides a convenient method of collecting specimens for the preparation of stained smears. This technic, which is particularly effective for amebic trophozoites, should be used in conjunction with other procedures which are more effective in the recovery and identification of cysts.

3. A significant relationship between the presence of *E. histolytica* infections and outside toilet facilities was demonstrated. This correlation apparently was not related to the rural residence of the patients.

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EFFECTS OF GROWTH *IN VITRO* WITH SELECTED MICROBIAL ASSOCIATES AND OF ENCYSTATION AND EXCYSTATION, ON THE VIRULENCE OF *ENDAMOEBA HISTOLYTICA* FOR GUINEA PIGS

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After more than 50 years of amebiasis research, it is not known whether *Endamoeba histolytica* is in itself capable of producing disease or whether its ability to produce symptoms is dependent upon the activity of associated organisms. These questions have not lent themselves to scientific investigation because of the following complicating factors: the intestinal flora of experimental hosts has provided a factor that could not be overlooked in attempts to ascertain the effects of the ameba on tissues of the hosts. Furthermore, the presence of bacteria in all cultures of the ameba has provided an almost insurmountable complicaton to the preparation of a sterile amebic inoculum for use in such studies.

Recently, however, the rearing of experimental hosts free of all demonstrable microorganisms (Reyniers and Trexler, 1943) has provided animals which may eventually be suitable for such an investigation. The cultivation of *E. histolytica* in the absence of bacteria with *Trypanosoma cruzi* (Phillips, 1950) has provided a source of amebic inoculums which may be adequate for such studies. Hence it appears that recent advances have paved the way for an effective study of the problem. However, a further complication has appeared in that nearly all attempts to induce amebiasis with *E. histolytica* cultivated with *T. cruzi* have been without success. A strain of the ameba that produces acute amebiasis in rabbits and guinea pigs when the amebas are cultivated with bacteria seems to lose its virulence when cultivated with the trypanosome (Luttermoser and Phillips, 1952). The present report describes methods employed in attempts to elucidate this phenomenon. *E. histolytica* derived from such cultures at various indicated intervals following isolation from bacterial associates and at various intervals following encystation and excystation have been inoculated intracecally into guinea pigs maintained on a sterile experimental diet.

EXPERIMENTAL PROCEDURES

The 200 strain of *E. histolytica* (Tobie, 1949) was used in all experiments. Cultures of the ameba with *T. cruzi* were maintained by methods which have been previously described (Phillips, 1951). Amebic inoculums were prepared from the cultures by the following procedures: culture tubes were immersed in iced water for two to three minutes to free amebas from the walls of the tubes. The petrolatum seal was removed with a wire loop and the culture fluid agitated with a Pasteur pipette fitted with a rubber bulb. The fluid containing the suspended amebas was then transferred to 50 ml. centrifuge tubes each of which

[1] Laboratory of Tropical Diseases.

contained several loopfuls of rice flour; the rice flour prevented the amebas from becoming packed in an agglutinated mass during centrifugation. Following centrifugation for three minutes at 2,000 r.p.m., the supernate was removed to a depth of 5 ml., counts of the amebas were obtained with a hemocytometer, and sterile medium was added in a quantity sufficient to render the concentration of amebas approximately 100,000 per ml.

The isolation of trophic amebas from an association with bacteria to medium with *T. cruzi* was facilitated with the aid of antibiotics. Minimal inoculums of the ameba (0.1 ml.) were transferred into ameba-trypanosome medium containing penicillin G, 10,000 units/ml., and streptomycin 0.010 gm./ml. At the time of the first serial transfer the tubes were agitated and inverted allowing the culture fluid containing the antibiotics to be removed and discarded. Ten ml. of chilled, sterile ameba-trypanosome medium was then added to each tube to remove amebas from the walls of the tubes. One ml. of the resultant suspension of amebas was then transferred to each new tube of ameba-trypanosome medium. Cultures which were demonstrated by standard bacteriological procedures to be free from bacteria after the second serial transfer were used as the inoculum for experimental hosts. The method for microisolation of sterile cysts into the medium with *T. cruzi* has been previously described (Phillips and Rees, 1950).

The guinea pigs used in all experiments were from the N.I.H. Hartley #1 colony. Animals were obtained at the age of two weeks and maintained on a Quaker Oats-Purina laboratory chow diet for an additional week prior to their inoculation. The diet was prepared by the following formula: Quaker Oats 50 gm., Purina laboratory chow 100 gm., and water 500 ml. The diet was autoclaved for 45 minutes at 15 lbs. pressure and given to the animals *ad libitum*.The diet was supplemented with ascorbic acid which was added in quantities adequate to ensure that each animal obtained a minimum of approximately 10 mg. each day. The reason for sterilization of the diet was in order to conform as nearly as possible to procedures employed in the preparation of diets for germ-free animals at the the Lobund Institute, University of Notre Dame, since information obtained thereby might be useful in future studies with the germ-free hosts.

The formula for the Quaker Oats-Purina diet was developed as a result of considerable preliminary investigation. Corn, oats, wheat, and corn meal were sterilized and fed singly and in combinations to various groups of animals. In addition, each of the foregoing preparations was used in combination with Purina laboratory chow in varying concentrations. The diets were evaluated on the basis of the following criteria: palatability to the host, nutritional adequacy as judged by the appearance of the guinea pigs, and upon ability to permit the development of amebiasis within the hosts. Amebic inoculums for these preliminary studies were derived from cultures of the ameba with bacteria since the ameba-trypanosome cultures had failed to exhibit any virulence for the animals at the time these studies were initiated. The Quaker Oats-Purina diet was selected as the diet of choice on the basis of all three criteria.

The procedure of inoculation was similar to that used in previous investigations (Luttermoser and Phillips, 1952). Laparotomies were performed under

ether anesthesia and 1 ml. of the ameba suspension containing approximately 100,000 amebas was injected directly into the cecum of each animal.

Animals that survived for 21 days were sacrificed and examined for macroscopic lesions of the intestinal wall and for amebas in fecal material taken from several areas of the colon and cecum. Animals dying prior to the end of the 21-day observation period were similarly examined at the time of death. Animals dying from causes other than amebiasis were not included in the experimental data; the number of animals dying from other causes however, was less than one per cent of the total employed. The results were graded N, P, 1, 2, 3, and 4 by the following criteria: N—negative for amebas and lesions; P—positive for in amebas the lumen only; 1—one small area (less than 10 mm. in diameter) exhibiting early lesions; 2—several large ulcers on cecal wall involving not more than one-half of the cecum; 3—many large ulcers on cecal wall involving more than one-half of the cecum; and 4—area of ulceration involving entire cecal wall (usually a confluency of lesions) and extending into the colon.

The first experimental series utilized an inoculum of amebas that had been associated with *T. cruzi* in culture for a period of 5 months at the beginning of the experiment. In the second experimental series amebas were derived from ameba-trypanosome cultures at intervals beginning one week after their isolation, by treatment with antibiotics, from a culture with a mixed bacterial flora. After 10 weeks association with the trypanosome in culture the amebas were utilized to initiate two other series of cultures, one in which they were associated with the Shaffer-Frye Streptobacillus (Shaffer, Ryden and Frye, 1948) and *Clostridium perfringens*, and the other in which they were associated with the N.R.S. bacterial flora. After 20 days with these bacterial associates during which time occasional cysts were observed in both series of cultures, the amebas were isolated into medium with *T. cruzi*, in one case, series 2, with antibiotics and in the other, series 4, by microisolation. These were utilized as the inoculums for other series of animals at intervals beginning one week following isolation from the respective bacterial associates. In addition, the cultures of *E. histolytica* with the N.R.S. bacterial flora were maintained and were induced to encyst by the method of Dobell and Laidlaw (1926). Cysts derived from these cultures were harvested every other week and microisolated into medium with *T. cruzi*. Amebas from the resultant cultures were used as the inoculum for a fifth series of animals beginning from one to three weeks following isolation with the trypanosome.

EXPERIMENTAL DATA

The results of the intracecal inoculation of guinea pigs with *E. histolytica* derived from cultures of the ameba with *T. cruzi* are shown in Table 1. The derivation of cultures used in the various experiments is shown in Figure 1. These data show that the 200 strain of *E. histolytica*, which exhibits a high degree of virulence for certain experimental hosts when the amebas are derived from cultures with bacteria (Luttermoser and Phillips, 1952, and Tobie, 1949), apparently undergoes an alteration of its virulence upon prolonged association

TABLE 1

Results of the intracecal inoculation of three weeks old guinea pigs with approximately 100,000 E. histolytica *trophozoites derived from ameba-trypanosome cultures*

SERIES NUMBER	WEEKS FOLLOWING A-T ISOLATION[1]	NUMBER OF ANIMALS INOCULATED	RESULTS[2] Number of animals					
			N	P	1	2	3	4
1	21 to 49	364	222	142	0	0	0	0
2	1	14	0	0	2	1	4	7
2	3	12	0	2	2	1	3	4
2	5	8	2	1	2	1	0	2
2	6	10	5	3	1	0	0	1
2	7	10	4	3	0	0	2	1
2	8	10	7	3	0	0	0	0
2	12	10	6	4	0	0	0	0
3	1	10	1	3	1	3	0	2
3	2	15	4	4	2	2	1	2
3	3	15	7	5	0	1	2	0
3	4	15	5	6	1	3	0	0
3	5	15	9	5	0	0	1	0
3	7	15	9	6	0	0	0	0
4	3	15	3	5	2	1	1	3
4	4	15	8	5	0	1	0	1
4	5	15	5	9	1	0	0	0
5	1	10	1	0	3	1	3	2
5	2	10	1	0	2	1	1	5
5	4	10	4	4	1	1	0	0
5	6	10	7	3	0	0	0	0

[1] Number of weeks *E. histolytica* was cultivated with *T. cruzi* prior to inoculation.

[2] N—Negative for ameba and lesions.
P—Positive for ameba in the lumen only.
1—Early lesions in area less than 10 mm. in diameter.
2—Several large ulcers but involving less than one-half the cecum.
3—Many large ulcers involving more than one-half the cecum.
4—Area of ulceration involving entire cecal wall and extending into the colon.

n culture with *T. cruzi*. For example, of 364 guinea pigs inoculated with amebas derived from cultures in which the ameba was cultivated with *T. cruzi* a minimum of 21 weeks prior to inoculation, none developed acute amebiasis. Nevertheless, 142 of the animals in the group harbored amebas in the intestine. The inoculations in other experimental series gave strikingly different results. For example, in series 2, each of 14 animals inoculated with amebas cultivated with *T. cruzi* but one week prior to inoculation developed acute amebiasis. Similar results were obtained in the same series with amebas having a three weeks cultural association with *T. cruzi*. However, when the isolation was five weeks old there began a progressive decrease in the virulence of the culture and it became avirulent for the animals at the end of the eighth week. In series 3, which utilized the ameba after transfer from an association with *T. cruzi* to two known species of bacteria followed by isolation again with *T. cruzi*, there was some

restoration of virulence. Six of 10 animals inoculated with the ameba 1 week after return to culture with *T. cruzi* developed acute amebiasis. Here again, however, there was a gradual decrease in virulence of the cultures that resulted in a disappearance of virulence after the seventh week. In series 4 and 5 the amebas were transferred from the trypanosome associate to the N.R.S. bacterial flora, encystation was induced, and the resultant cysts were microisolated into medium with *T. cruzi*. In series 4 the cultures were initiated from only 5 cysts and could not be used as inoculum until three weeks following isolation. Nevertheless, the results show that the virulence was somewhat enhanced. In series 5 the cultures were initiated by the microisolation of 100 cysts into medium with *T. cruzi* and used as inoculum for animals after only one week. Amebas derived from these cultures exhibited a high degree of virulence in that 18 of 20 animals inoculated

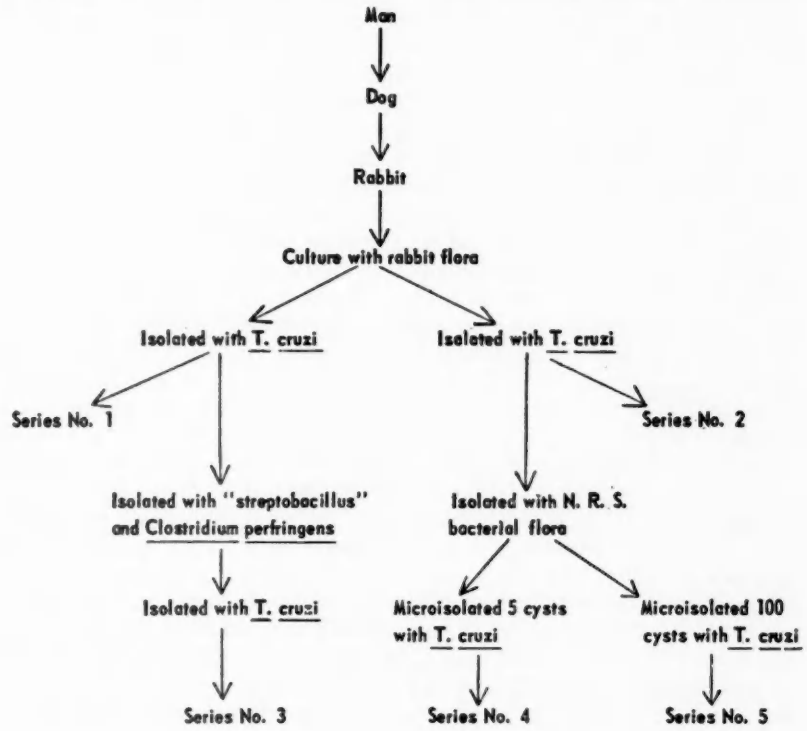

Figure I. Derivation of various isolations of strain 200 E. histolytica used in the various experimental series shown in table I. Series 1, 2, and 3 were isolated with the use of antibiotics; series 4 and 5 were isolated by microisolation.

FIG. 1

the first two weeks developed acute amebiasis. The diminution of the high virulence was similar however to that of the other series. After six weeks association with *T. cruzi* the ameba failed to produce acute disease in any of 10 animals which were inoculated.

DISCUSSION

As a result of the studies reported herein, methods have been devised which permit the preparation of bacteria-free amebic inoculums of high virulence for studies of amebic pathogenicity in germ-free hosts. Furthermore, the data are of interest since they show that alterations in the virulence of amebic cultures may be attributable to changes in the ameba *per se*. In a previous report (Luttermoser and Phillips, 1952) it was shown that the cultural associates exerted considerable influence upon the virulence of a strain of *E. histolytica*. However, it was not shown unequivocally that such influence was exerted on the ameba *per se* as opposed to a theory that such influence may be attributable to the flora which accompanies the inoculum into the host.

The data of the present studies provide not only confirmation that the cultural associates exert considerable influence upon the pathogenicity of *E. histolytica*, but they show, in addition, that such influence may be inherent in a direct change in pathogenicity of the ameba. When the amebas were associated in culture with *T. cruzi* for a period of five months they became avirulent for experimental hosts. However, when the amebas were returned to culture with bacterial associates followed by reisolation with *T. cruzi* their virulence was restored and was maintained for a period of three to five weeks. A similar restoration of virulence was noted when the amebas were induced to encyst and the resultant cysts used to initiate cultures of the ameba with *T. cruzi*. The effects of the *T. cruzi* associate upon the virulence of the ameba was further demonstrated in that there was a gradual diminution in the virulence of these ameba-trypanosome cultures which progressed until they became entirely avirulent for the experimental hosts. Since the data show that these cultures remained infective for a considerable number of animals, as evidenced by the demonstration of amebas in the intestine at the end of the observation period, the failure of the amebas to produce symptoms may be attributable only to changes in the pathogenicity of the ameba *per se*.

The identity of the factor(s) operative in the phenomenon of induced variable pathogenicity of *E. histolytica*, as demonstrated herein, remains somewhat obscure. Deschiens (1939, 1941) and Chang (1945) have observed a restoration of virulence of the ameba resulting from induced encystation *in vitro*. Similarly, each restoration of virulence in the present studies occurred following return of the ameba to culture with bacteria wherein some encystation was observed. The apparent failure of the ameba to produce cysts in culture with *T. cruzi* suggests a possible explanation for their loss of virulence in the ameba-trypanosome cultures. However, although the data strongly suggest that periodic encystment may be essential to maintenance of virulence of the ameba *in vitro*, and that encystation was responsible for the restored virulence observed in the present

studies, unequivocal proof for such an hypothesis is not presented. Changes in the nutritive habits of the ameba, the oxidation reduction potential of the cultures, and many other factors inherent in such radical environmental changes may conceivably influence the restoring and/or maintaining of the virulence of *E. histolytica in vitro.*

SUMMARY

E. histolytica derived from cultures with *T. cruzi* were inoculated intracecally into guinea pigs at various intervals following isolation of the ameba with the trypanosome associate. The results showed that prolonged cultivation of the ameba with *T. cruzi* resulted in a complete loss of its virulence. However, the virulence was restored by returning the ameba to culture with selected bacteria followed by reisolation with *T. cruzi*, or by initiating ameba-trypanosome cultures from cysts produced in culture with the N.R.S. mixed bacterial flora. Cultures having their virulence thus restored retained such virulence for periods of three to eight weeks.

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THE DONALDSON STRAIN OF MALARIA

1. History and Characteristics of the Infection in Man

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HISTORY OF THE STRAIN

In June of 1949 a strain of malaria was recovered from a service man who had returned to the United States from the Pacific area some three and a half years previously. The strain was designated as the "Donaldson Strain" after its original carrier. This carrier was experiencing his fourth clinical episode of malaria when the parasite was isolated. He had served as an officer in the Sanitary Corps of the Army in the West Pacific areas from February, 1944, until September, 1945. Apparently the infection originated either in the Philippines or the Admiralty Islands, with the best chance of acquiring the infection being on Luzon in the Philippine Islands. The officer was subjected to heavy suppressive doses of quinacrine (Atabrine) during his entire stay in the Admiralty Islands and the Philippines. The suppressive quinacrine was discontinued on or about September 2, 1945, when he left Luzon for Japan.

On January 1, 1946, after he had returned to the United States, the officer suffered his first attack of malaria. This attack subsided after a course of sulfadiazine. A relapse was experienced in November of 1946, which was terminated with quinacrine. Further parasite recurrences in 1947 and early 1948 were not accompanied by frank malarial symptoms and were not treated, but a symptomatic relapse in August, 1948, was again terminated with quinacrine. Another relapse occurred in June, 1949, at which time the strain was obtained by us for study. This relapse was terminated with chloroquine and no further parasite recurrences have been experienced by the original carrier.

Since its recovery in June, 1949, about 125 neurosyphilitic patients have been inoculated with this strain. Although the strain was first considered to be one of *Plasmodium vivax*, it was noted during the first few passages that it differed considerably from the strains of *vivax* being used in these laboratories. In many ways the characteristics of the strain closely simulated those reported for *P. ovale*, and for this reason it was subjected to intensive study.

The following report will present the clinical and parasitological characteristics of the infection in an effort to establish the identity of the strain. Subsequent reports will consider the parasite morphology and the characteristics of the infection in the mosquito host.

[1] The authors wish to express their gratitude to Dr. Alan W. Donaldson, Sr. Scientist, U. S. Public Health Service, not only for providing the parasite, but also for an expert appraisal of the origin of the infection.

METHODS

The infection was studied in about 125 neurosyphilitics undergoing therapeutic malaria. Inoculations were done either by the transfusion of infected blood or by the feeding of infected mosquitoes. Daily blood smears of the Earle-Perez (1932) type were initiated immediately in blood-inoculated cases, and after six days in sporozoite-induced cases. Parasite densities were determined and recorded in terms of cubic millimeters of blood. These daily blood films were continued until the patient had experienced 30 consecutive negative days, after which time twice-weekly observations were continued for a period of a year, in most cases. The twice-weekly blood films were ordinary thick-thin blood smears, and amounts of blood equivalent to about 0.1 cmm. were examined on each slide. In the event of parasite recurrences, daily observations were resumed.

An effort was made to record complete clinical data for each case. Oral temperature readings were initiated at the same time as the blood films and were done at four-hour intervals until first elevation of 100°F. or more was noted. Hourly temperatures were then recorded until the temperature again returned to below 100°F. This schedule was continued during the clinical stages of the infection.

Most of the strain characteristics of the Donaldson strain are compared directly with two strains of *P. vivax*, the Chesson and Saint Elizabeth strains. Observations on these two strains were conducted in the same way as those on the Donaldson strain. Since these two *vivax* strains have been used in these laboratories for a number of years their infection characteristics are well known by us.

OBSERVATIONS

Prepatent and incubation periods. For the purpose of this section only sporozoite-inoculated cases are considered. Table 1 summarizes these periods in the Donaldson strain and two strains of *P. vivax*.

In 37 patients inoculated with the bites of 5 to 35 infected mosquitoes (mean

TABLE 1

Early activity of mosquito inoculated Donaldson strain as compared to the Chesson and St. Elizabeth strains of P. vivax

	NO. OF CASES	NO. OF INFECTIVE BITES			PREPATENT PERIOD IN DAYS			INCUBATION PERIOD IN DAYS		
		Range	Mean	Median	Range	Mean	Median	Range	Mean	Median
Donaldson strain	37	5–35	15.1	11	12–20	15.3	15	14–26	17.7	17
Chesson*	82	2–50	13.9	12.5	9–15	11.8	12	10–19	13.7	13
Chesson†	96	10	10	10	9–16	11.8	11	10–17	12.2	12
St. Elizabeth‡	50	10	10	10	11–18	13.8	14	8–18	14.7	?

* Personal observations.

† Coatney, Cooper and Young, 1950.

‡ Coatney, Cooper, Ruhe, Young and Burgess, 1950.

TABLE 2

Clinical activity of Donaldson strain as compared to Chesson and St. Elizabeth P. vivax

	NO. OF CASES	NO. OF PAROXYSMS			MAX. PARASITES/CMM.			MAX. FEVER MEAN	% PARTIAL SUPPRESSION NECESSARY	% EARLY TERMINATION NECESSARY
		Range	Mean	Median	Range	Mean	Median			
Donaldson strain	103	1–22	8.1	8	464–25,940	8,376.9	7,132	105.2	1.9*	1.9
Chesson	100	3–27	13.7	13	4,944–126,000	39,889.9	35,280	105.3	61.0†	87.0‡
St. Elizabeth	100	3–28	19.1	21	4,100–65,000	15,506	13,888	106.0	57.0	59.0

* Suppression with X gr. quinine terminated infection.

† Suppression with sodium bismuth thioglycollate, quinine, or both.

‡ Termination before cessation of clinical activity.

number of bites, 15.1) parasites were first detected 12 to 20 days following exposure. The mean prepatent period was 15.3 days and the median 15 days. First oral temperatures of 100°F. or above were seen 14 to 26 days after infective bites, with a mean of 17.7 days and a median of 17 days.

The mean prepatent period in the Donaldson strain (15.3 days) exceeded that in the two strains of *P. vivax* (11.8 and 13.8 days). An even more pronounced difference was seen in the mean incubation period, with the Donaldson strain exceeding the others by three to four days.

The Donaldson strain infected both white and Negro patients, with no apparent difference in the prepatent or incubation periods, or in the subsequent clinical activity.

Clinical activity. Table 2 presents some of the characteristics of the Donaldson strain infection which are related to the clinical period. Similar information on the Chesson and St. Elizabeth strains of *vivax* is included for comparison.

The Donaldson strain is characterized by a mild, short clinical period. There is an average of only 8.1 paroxysms per attack, in contrast with 13.7 for Chesson and 19.1 for St. Elizabeth. The mean maximum parasitemia of about 8,377 per cmm. in the Donaldson strain is also significantly lower than that seen in the two strains of *P. vivax* (39,890 in Chesson and 15,506 in St. Elizabeth). The mean maximum temperatures show little difference in the three strains.

In addition to the lower numbers of paroxysms and maximum parasitemias it should be noted that in only two cases of the Donaldson strain was it deemed necessary to suppress or terminate the infection before spontaneous completion of the clinical activity. In the Chesson strain (Table 2) partial suppression with sodium bismuth thioglycollate and/or quinine was necessary in 61 per cent of the cases, while the attack was terminated prior to the end of primary clinical activity in 87 per cent of the cases. This is not to say that the clinical activity would not have ended spontaneously, but the mean number of paroxysms in the Chesson strain is lowered considerably by this early termination. The same early termination was used in about 59 per cent of the St. Elizabeth cases, similarly affecting the number of paroxysms.

The use of partial suppression affected the maximum parasitemias in much the same way, undoubtedly prejudicing the mean maximum parasitemias of the two strains of *vivax* to a somewhat lower than normal figure.

Periodicity and synchronicity of the infection. The paroxysms in the Donaldson strain were quite variable in periodicity. The strain showed a pronounced tendency to produce multiple parasite broods, frequently resulting in quotidian fevers. Infrequently the fevers would be tertian throughout the course of the infection, but generally the tertian cycle would be preceded and/or followed by a quotidian periodicity. Quite often the quotidian fevers persisted through the whole clinical course. Efforts to convert the quotidian cycle to tertian by use of sodium bismuth thioglycollate generally met with failure, since doses of 0.05 to 0.1 grams intramuscularly would terminate the infection, or completely suppress it for a number of days.

The periodicity of the infection was calculated using the intervals between tertian fever peaks as reference points. Table 3 summarizes the length of 237 of these paroxysmal intervals, and compares them with similar findings in the two *vivax* strains. It is apparent that the mean length of 49.5 hours is considerably higher than those found in the Chesson and St. Elizabeth strains of *P. vivax*, 44.6 and 43.4 hours, respectively.

Response to antimalarial treatment. This strain of malaria seems to be extremely sensitive to all antimalarial drugs tried. Because of the benign character of the strain, opportunities to observe treatment of clinical attacks have been comparatively few. Table 4 summarizes the results of treatment with several drugs given during the primary attack. Observations on the Chesson strain of *vivax* are included for comparison.

It is apparent that in all cases the parasitemias of the Donaldson strain were reduced more rapidly by the same or lower dosages of drug than were those of Chesson *vivax*. Two cases of Donaldson treated with 10 grains (0.65 grams) of quinine were completely cleared of parasites in four days. In Chesson cases treated with such low doses complete parasite clearing did not ensue without further medication. Even therapeutic dosages of 120 grains (30 per day for 4 days) did not clear Chesson parasitemias as rapidly as did a 10 grain single dose in Donaldson.

One case which received only 0.05 grams of sodium bismuth thioglycollate showed a parasite reduction of 93 per cent in 24 hours. The greatest reduction

TABLE 3

Length of paroxysmal intervals in the Donaldson strain and two strains of P. vivax

	NO. OF INTERVALS	RANGE IN HOURS	MEDIAN LENGTH	MEAN LENGTH
Donaldson	237	34–61	49	49.5
Chesson	149	39–49	44	44.6
St. Elizabeth*	401	31–57	43	43.4

* Data from Young, 1944.

TABLE 4

Results of treatment of the Donaldson strain with four antimalarial drugs compared with the Chesson strain of P. vivax

DRUG	STRAIN	DOSAGE IN GRAMS	NO. OF CASES	AVERAGE PER CENT REDUCTION OF PARASITEMIAS IN				
				24 hrs.	48 hrs.	72 hrs.	96 hrs.	120 hrs.
Sodium bismuth thioglycollate*	Donaldson	0.05–0.1	3	70.6	82.1	96.3	85.0	98.5
	Chesson	0.1–0.2	19	42.9	46.2	65.8	61.0	62.8
Quinine sulfate	Donaldson	0.65–1.3	3	51.7	92.5	98.9	99.9	100.
	Chesson	0.65–7.8	10	48.9	68.2	73.7	80.3	85.5
Chloroquine†	Donaldson	1.5	5	83.8	96.3	100.	100.	100.
	Chesson	1.5	10	74.9	98.4	99.6	99.9	100.
Chlorguanide	Donaldson	0.1	1	68.6	83.7	99.2	99.9	99.9
	Chesson	0.1–0.4	3	32.0	82.1	98.0	99.5	99.8

* Intramuscular. All others given orally.

† Chloroquine regimen: 0.9 g. day 1; 0.3 g. day 2 and 3.

seen in 19 cases of Chesson *vivax* after 0.1 grams was 92 per cent. In one of the Donaldson cases receiving 0.1 grams the parasites were completely cleared in four days; in no case of Chesson similarly treated did this occur.

The action of chloroquine and chlorguanide is similar on both strains, although with both drugs there were more Donaldson parasites removed in 24 hours than Chesson.

Persistence of parasitemias and relapse. In untreated cases asymptomatic parasitemias continue for considerable periods of time. In this respect there is little difference between blood- and sporozoite-inoculated cases. Table 5 summarizes the total duration of parasitemias in 35 untreated infections.

In these cases the clinical period terminated after 8 to 26 parasite days with an average duration of 16.9 parasite days. Following cessation of symptoms the parasitemias continued in the peripheral blood for 11 to 89 days, with an average duration of 37.5 days. These asymptomatic parasitemias were generally of a low grade and intermittent. The total duration of parasitemias in untreated cases averaged 54.5 days.

There was very little opportunity to observe the Chesson strain of *vivax* in regard to duration of asymptomatic parasitemias. In most cases infections with this strain were terminated by drug before the cessation of symptoms or very shortly

TABLE 5

Persistence of parasitemias in the Donaldson strain in spontaneously terminated attacks

	MOSQUITO INOC.	BLOOD INOC.	TOTAL
No. of cases	11	24	35
Range in days	29–91	28–103	28–103
Mean No. of days	53.2	55.0	54.5
Median No. of days	51	54	53

thereafter. Only a single case was allowed to run to completion of primary parasitemias. This was in a Negro patient and was of 122 days duration. Two other cases in Negores showed asymptomatic parasitemias of some length, but these were terminated by drug after a total duration of 40 and 100 days, before parasitemias disappeared spontaneously. A single white patient showed continuous parasitemias for as long as 71 days, but termination by drug at this point prevented determination of the normal duration. It is thought that in all infections with this strain of *vivax* in which the clinical attack terminates spontaneously, asymptomatic parasitemias would continue at least as long as those of the Donaldson strain. It is interesting to note that the larger number of spontaneously terminating Chesson infections was in Negroes, who are usually immune to this strain.

Symptomatic relapses are infrequent in the Donaldson strain following termination of the sporozoite-induced primary attack with chloroquine or following spontaneous termination. In our experience, in only two of 38 mosquito-inoculated cases have symptomatic relapses occurred, and these were following termination of the primary attack with chloroquine during the first few days of parasites and before the patient had experienced more than two paroxysms. These cases experienced mild, spontaneously terminating symptomatic relapses 148 and 235 days after termination of the primary attack. One of these relapses received a course of chloroquine-primaquine after cessation of symptoms and had no further parasite recurrence. The other was treated with chloroquine alone and experienced low-grade asymptomatic parasitemias 152 days later.

Asymptomatic parasitemias tend to recur quite frequently, but with no orderly pattern, for periods of six to eight months following termination of the primary attack. These parasitemias are generally of a very low grade and may persist as long as one or two weeks.

The infrequency of symptomatic relapses in the Donaldson strain is in contrast to the almost invariable occurrence of such relapses in sporozoite induced cases of St. Elizabeth and Chesson *vivax* not treated with curative drugs.

Immunity studies. One inoculation with the Donaldson strain seems to confer immunity to reinfection with the same strain. Table 6 presents some of the immunological relationships of the Donaldson strain to Chesson *P. vivax*.

TABLE 6

Immunity: Donaldson vs Chesson

	DONALDSON STRAIN FOLLOWING SINGLE CHESSON	DONALDSON STRAIN FOLLOWING MULTIPLE CHESSON	TOTAL DONALDSON FOLLOWING CHESSON	DONALDSON CONTROLS
No. of cases	5	10	15	103
Paroxysms				
Range	4–8	4–9	4–9	1–22
Mean	6.0	5.7	5.8	8.1
Max. parasitemias				
Range	1,584–19,440	5,088–21,780	1,584–21,780	464–25,940
Mean	5,704.8	8,588.4	7,915.7	8,314.0

It is apparent that the Donaldson strain infection following Chesson infection is of only slightly lesser intensity than the controls. The maximum parasitemias average about the same, while the mean number of paroxysms is somewhat lower in the Chesson immune patients.

In five patients inoculated with Chesson *P. vivax* following a Donaldson strain infection, there was no apparent modification of the ensuing infections. The mean number of paroxysms (15) actually exceeded the normal for that strain (Table 2) and the average maximum parasitemia (29,556 per cmm.) was insignificantly lower than normal.

The Donaldson strain had no apparent cross immunity with *P. falciparum* or *P. malariae*.

The strain seems to be equally infective to Negroes and whites; no immunes were encountered in 55 Negro patients inoculated. This is in contrast to immunity that most Negroes show to the *vivax* strains. In only one case was a Negro patient susceptible to both Donaldson and Chesson strains. Six Negro patients found immune to the Chesson strain subsequently were susceptible to the Donaldson strain, and infections in these patients were above normal in intensity, with averages of 8.5 paroxysms and 11,533 parasites per cmm. maximum (cf. Table 6).

DISCUSSION

The mean prepatent period of sporozoite induced Donaldson strain (15.3 days) compares favorably with this period in *P. ovale*. James *et al.* (1932) report the period for *ovale* as 14 or 15 days, while Sinton *et al.* (1939 B) found a prepatent period of 15 days. The two strains of *vivax* used for comparison had mean prepatent periods of 11.8 and 13.8 days, and most of the literature describes the prepatent period of *P. vivax* as normally falling between 11 and 13 days.

The literature reveals that the clinical activity of the Donaldson strain very closely simulates that of *P. ovale*. All authorities agree that the *P. ovale* infection is characterized by a mild clinical course consisting of small numbers of paroxysms not accompanied by the typical rigors of the *vivax* type. This description would apply also to the Donaldson strain. Our data on the severity of rigors in the Donaldson strain are not exact, but the general impression is that they are much milder than those experienced with *vivax*.

James, Nicol and Shute (1949) found that only about 15 per cent of 197 patients with *P. ovale* experienced 10 or more paroxysms in which the peak temperature exceeded 103°F. In the Donaldson strain the mean number of paroxysms with a temperature peak above 100°F. was 8.1, and in only about 25 per cent of all cases were there 10 or more paroxysms exceeding 100°F. Only 10 per cent of the cases experienced 10 paroxysms exceeding 103°F.

There is general agreement that the density of parasites in *P. ovale* is usually lower than in *vivax*. Sinton *et al.* (1939 B) and James *et al.* (1949) report wide variation in maximum densities, the former reporting from about 125 to 20,000 per cmm. and the latter from about 500 to 100,000 per cmm. In the Donaldson strain the maximum density ranged from 464 to 25,940 per cmm.

Characteristically, primary cases of *P. ovale* terminate spontaneously in a large

majority of uncomplicated infections. This fact has been reported by all who have had experience with the infection, either naturally or artificially acquired. Similarly, nearly all of the Donaldson strain cases also terminated without the aid of drugs, although as a rule asymptomatic parasitemias continued for some time.

The average duration of parasitemias of 37.5 days after cessation of symptoms is somewhat higher than that reported in the literature for *ovale*. James *et al.* (1949) note that in 28 patients (presumably out of a total of 193) the infection persisted during this "terminal" period from 20 to 30 days. Sinton *et al.* (1939 B) noted an average total duration of parasite incidence of 25.0 days in 45 cases receiving no treatment. The reason for the shorter duration in these cases probably lies in the method of enumerating parasites. The methods of Sinton and of James seen to preclude diagnosis of parasitemias lower than 125 to 500 per cmm., while our methods detect densities as low as 10 per cmm. Sinton *et al.* (1939 B) note that careful examinations of thick films revealed parasites in four *ovale* infections as late as days 53, 54, 82, and 93.

James *et al.* (1949) found no relapses in 36 sporozoite induced *P. ovale* infections, although 14 of these cases received no specific treatment for the primary attack. Sinton *et al.* (1939 A) reported only one true relapse in 22 sporozoite induced cases of *P. ovale*, although they noted the presence of parasites for several months after untreated primary attacks. Wilson and Wilson (1935) noted recurrence of *ovale* parasites at low levels in several autochthonous cases for as long as a year of observation. In a number of cases *ovale* infection has been detected in patients removed for some time from the endemic area, but the status of the infection in these cases is somewhat in doubt. It has been suggested (James *et al.*) that they may represent "recrudescences of the primary attack"; it seems to us that they may also possibly represent a delayed primary attack.

Only two true relapses were seen in 38 Donaldson strain cases, and these were mild clinically and terminated spontaneously in a fairly short time. Recurrence of asymptomatic parasitemias was seen quite frequently, although they persisted for only a few days at a time and were at very low levels. It is interesting to note, however, that the original infection was recovered nearly four years after the carrier had left the endemic area and suppressive drug was halted. During that time there were four episodes of malaria. Therefore, unless the patient had infections representing a mixture of several species, it must be assumed that the strain will relapse after considerable periods.

James *et al.* (1949) found that the primary attack of ovale malaria began with a period of irregularly remittent fever in about 50 per cent of the cases, followed by a quotidian periodicity in 26 per cent of the cases. Our findings with the Donaldson strain approximated this, with the exception that about one-half of our cases had an initial intermittent periodicity which was quotidian in nature. Even in cases where the initial periodicity was tertian, there was a tendency to convert to quotidian early in the primary attack.

Schizogony in *P. ovale* is generally said to be effected in 48 hours. The Donaldson strain apparently requires somewhat longer, with the mean paroxysmal inter-

val being 49.5 hours. It is interesting to note that the *P. ovale* fever chart reproduced by James *et al.* (1949) in Boyd's "Malariology" (Vol. 2, p. 1048) shows only one interval between fever peaks as short as 48 hours. The other seven intervals appear to range between about 52 and 56 hours each. In two temperature charts of James *et al.* (1932) eight paroxysmal intervals appear to vary from 44 to 52 hours, averaging 49.5 hours in length. Although *P. vivax* is also described as having a 48-hour periodicity it is perhaps significant that practically all strains which have been studied carefully show shorter periods. Young and associates (1944, 1947) noted periodicities averaging from 41.5 to 45.1 hours in eight foreign and domestic strains of *P. vivax*.

P. ovale is known to respond rapidly to small doses of quinine and quinacrine. Our experiences with the Donaldson strain have indicated that it is very easily eliminated with small quinine dosage, and is quite sensitive to sodium bismuth thioglycollate, a drug often used for mild suppression of therapeutic *P. vivax* infection.

P. ovale infection seems to confer a high degree of immunity against heterologous strains of the same species, but may be successfully inoculated into persons immune to the other three human plasmodia (Sinton *et al.* 1939 A). Apparently mixed infections, with *P. ovale* accompanying one of the other species, are quite common in endemic areas. It has not been possible to do cross-immunity studies between *P. ovale* and the Donaldson strain, but the Donaldson strain apparently confers no immunity to the other three species, nor does previous experience with these species materially affect susceptibility to the Donaldson strain. Sinton *et al.* (1939 A) note the susceptibility of a Negro patient (previously resistant to the other human plasmodia, as well as *P. knowlesi*) to *P. ovale*, and suggest that Negroes may show less racial immunity to *ovale* than to other strains of *Plasmodium*. On the basis of our experience, this observation might also be extended to the Donaldson strain, another indication of its similarity to *P. ovale*.

SUMMARY

A strain of malaria parasite was recovered from a man who had served as an Army officer in the West Pacific during World War II. This strain, termed the Donaldson strain, was studied in about 125 neurosyphilitics undergoing therapeutic malaria. Both sporozoite induced and blood induced infections were included, the former composing about one-third of the cases. Similar studies were done on two strains of *P. vivax* for comparison of strain characteristics.

In sporozoite induced Donaldson strain the mean prepatent period was 15.3 days and the incubation period 17.7 days. The strain was characterized by a short, mild primary clinical attack, with an average of only 8.1 paroxysms per attack and a mean maximum parasitemia of 8,314 per cmm. Termination of the clinical attack was almost invariably spontaneous. The mean paroxysmal interval, indicative of the periodicity of the strain, was 49.5 hours. The strain was characterized by a tendency to produce multiple parasite broods results in frequent quotidian periodicity.

The infection was very sensitive to antimalarial drugs and easily eradicated

with small amounts of quinine or sodium bismuth thioglycollate, as well as with standard dosages (1.5 g.) of chloroquine.

Untreated parasitemias persisted for considerable periods of time, with an average total duration of 54.5 days. Symptomatic relapses occurred in only two cases, and these were following very early termination of the primary attack with chloroquine. The clinical relapses were mild and terminated spontaneously after only a few febrile episodes.

The Donaldson strain is equally infective to Negroes and whites. One Donaldson strain infection seems to confer immunity to reinfection with the same strain, but not to the other species of *Plasmodium*. Previous infections with *P. vivax*, *P. falciparum*, or *P. malariae* do not significantly affect subsequent Donaldson strain infections.

The characteristics of the Donaldson strain are compared with those of the Chesson and St. Elizabeth *vivax* strains and with *P. ovale*. It appears that the Donaldson strain resembles *P. ovale* more than it does *P. vivax*.

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THE DONALDSON STRAIN OF MALARIA

2. Morphology of the Erythrocytic Parasites

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The history and characteristics of human infection of the Donaldson strain of malaria have been described in a previous report (Jeffery, Young and Wilcox, 1954). It is apparent that the infection differed markedly from that characteristic of *Plasmodium vivax*, and appeared to be quite similar to *P. ovale*.

This report, the second in the series on the Donaldson strain of malaria, will present the results of morphological studies on the erythrocytic parasites to further establish the identity of the strain as one of *P. ovale*.

MATERIALS AND METHODS

The Donaldson strain was compared morphologically with two known strains of *vivax*, St. Elizabeth and Chesson. Parasitemias studied were usually around 5,000 per cmm. or more. Smears were taken routinely each morning on every patient and at shorter intervals on a selected group of patients for periods of time covering three successive paroxysms. Thin and thick smears were made but the thin films were used for most of the determinations.

Since the method of staining and materials used account for many variations in appearance of stained blood smears, particular care was exercised in each step of the procedure, and every attempt was made to assure good detail in each part of the parasite and comparable results in all respects. The consistency of results obtainable prompted the use of the long method of Giemsa staining (Wilcox, 1950). For pigment studies, smears were stained by this same method with the addition to the stain solution of 0.5 per cent of Triton X-30 (Donaldson and Brooke, 1950), as we found that this addition makes the color appear somewhat lighter. The slides then were washed for an hour in neutral distilled water containing Triton to remove a great deal of the color of the cytoplasm, chromatin, and Schüffner's stippling and to make the pigment granules more prominent.

A planimeter, set for measuring in square inches, was used to measure the area of the camera lucida drawings. These drawings were made on paper ruled in inch squares, the sides of each square divided in tenths. Using the camera lucida with a 15× ocular and 90× objective and with the ruled paper placed on a board about $\frac{3}{8}$ inches thick, each $\frac{1}{10}$ of an inch on the paper equalled 1 micron of the stage micrometer. Hence the measurements made in square inches could be converted directly to square microns (1 sq. inch equalling 100 sq. microns), and the ruled paper acted as a visual check on the results of the measurements. Each cell and each parasite was traced 5 times with the planimeter. The reading was divided by 5 and multiplied by 100 to convert to square microns.

The parasites and cells chosen for study always lay in the parts of the smear where cells were evenly distributed and well separated so that there was quick drying without much chance of shrinkage and no crowding of cells in order that distortion of the size and shape would be limited and as nearly uniform as possible.

OBSERVATIONS AND RESULTS

Cell and parasite measurements. Measurements were made of 100 uninfected red cells and of an equal number of cells parasitized by each of three stages in the three strains of malaria parasite. These three stages were young rings, the binucleate schizont, and the mature schizont. Results of all measurements are summarized in Table 1.

The area of uninfected erythrocytes averaged slightly over 49 sq. microns for each strain.

There was little variation in area of ring stages in the three strains. The cells containing ring stages in both Chesson and St. Elizabeth strains showed considerable enlargement (10.3 and 11.5 per cent respectively) over the uninfected cell, while in the Donaldson strain the parasitized cell containing this stage measured the same as the nonparasitized cell.

With the binucleate schizont, the average infected cell of Chesson was increased in size by 52.9 per cent over the normal cell, that of St. Elizabeth by 44.0 per cent, and that of Donaldson by only 26.8 per cent.

The increase in size of the cell with the mature schizont over that of the normal cell was somewhat greater in each instance than with the binucleate schizont: 55.8 per cent in Chesson, 50.7 per cent in St. Elizabeth, and 27.4 per cent in Donaldson. It will be noted that the binucleate and mature schizonts of St. Elizabeth were considerably larger than those of Donaldson, while those of Chesson were even larger than the same stages of St. Elizabeth.

Using 1.0 as the normal cell, an estimate of the increase in size of infected cells (all measured stages combined) over the normal cells is as follows: Donaldson 1.18, St. Elizabeth 1.35, and Chesson, 1.40.

Merozoites were counted in 200 mature schizonts of each of the two known *vivax* strains and in 700 mature schizonts of the Donaldson strain. The results are

TABLE 1

Comparative areas in square microns of 3 strains—by planimeter measurements of camera lucida drawings (100 drawings for each figure given)

STRAIN	UNINFECTED CELL	YOUNG RINGS		BINUCLEATE SCHIZ.		MATURE SCHIZ.	
	cell	cell	parasite	cell	parasite	cell	parasite
Donaldson	49.3	49.3	6.5	62.5	27.2	62.8	30.0
St. Elizabeth	49.5	55.2	6.6	71.3	35.8	74.6	41.6
Chesson	49.3	54.4	6.4	75.4	41.6	76.8	45.5

TABLE 2

The percentage distribution of merozoite numbers in mature schizonts of the three strains

	NO. OF MEROZOITES (PER CENT)		
Strain	Chesson	St. Elizabeth	Donaldson
No. of segmenters	200	200	700
4			2.4
5			1.3
6			29.4
7			2.3
8			46.6
9		7.5	1.7
10	1.0	0.5	10.1
11	0.0	1.0	0.6
12	7.0	15.5	2.9
13	0.5	0.5	0.0
14	5.5	37.5	2.0
15	1.0	4.5	0.0
16	36.0	25.0	0.7
17	1.0	4.0	
18	19.5	1.0	
19	4.0	0.0	
20	12.0	3.0	
21	1.0		
22	9.0		
23	0.5		
24	2.0		
Av. no. merozoites	17.3	14.1	7.8

summarized in Table 2. The average number of merozoites for Chesson was 17.3, for St. Elizabeth, 14.1; and for Donaldson, 7.8. The percentages of segmenters containing over 12 merozoites were: Chesson, 92; St. Elizabeth, 74; and Donaldson, 3.

Other parasite observations. While no growing trophozoites of the Donaldson strain were chosen for measurement because of the difficulty of determining comparable ages of the three strains of parasite studied, these stages (Plate 2, Fig. 7–19) presented some of the most striking differences when compared with the known *vivax* strains (St. Elizabeth, Plate 2, Fig. 7–14, Chesson, Plate 1, Fig. 7–15). Beyond the ring stage the Donaldson parasite had a distinct tendency toward solid, compact arrangement of chromatin and cytoplasm with very little ameboid appearance. On the other hand, the older trophozoites of both Chesson and St. Elizabeth strains usually possessed the tenuous, irregular shapes and great variety of cytoplasmic pattern, often with large vacuoles, so characteristic of *vivax*. These bizarre shapes were even more pronounced in the Chesson than in the St. Elizabeth strain. The Donaldson trophozoite and immature schizont (Plate 2, Fig. 14; Plate 4, Fig. 24) were often rounded and in the center of the

cell. Only occasionally were band forms found in the Donaldson strain but these forms were found also in rare instances in both St. Elizabeth and Chesson infections, so no significance was attached to them.

There was no easily noted morphological difference between the Donaldson and *vivax* gametocytes except that the average sexual parasite and containing cell were usually smaller in the Donaldson strain. There was a great variation in size of gametocytes in both of the *vivax* strains, however, and differentiation of strains on the basis of gametocytes alone would be most untrustworthy. Female gametocytes of the Donaldson strain were often differentiated with difficulty from compact mature trophozoites. Male gametocytes, on the other hand, were usually easily determined.

In all three strains studied, pigment particles apparently increased in size and number, as the parasite grew. The pigment granules of the Donaldson strain may have been slightly larger than in the other strains, though even in lightly stained blood films it was difficult to be certain. There also appeared to be fewer pigment granules in the Donaldson strain than in the two *vivax* strains. No set pattern of pigment arrangement could be noticed in the three strains other than the clumping of pigment in the mature schizont. Occasionally a peripheral arrangement of pigment was seen in the Donaldson strain, but this was not a consistent characteristic. The color of the pigment in parasites of similar age and density and in masses of similar size seemed about the same in all three strains. In the carefully stained smears, it varied from the almost inapparent yellow haze in young forms through yellow brown into deep gray brown in the older parasites. Pigment granules or masses in sexual and asexual parasites of similar stages of growth were similar in size and color.

Morphology of infected erythrocytes. In the uncrowded thin portion of the blood smear the majority of all infected cells of three strains studied were more or less rounded, often with slight irregularities. In both Chesson (Plate 1, Fig. 12, 16) and St. Elizabeth (Plate 2, Fig. 8, Plate 4, Fig. 23) strains some infected cells were longer than wide or roughly elliptical. Oval shapes could be found on rare occasions in these two strains but too seldom for any significance. In the Donaldson strain, not only were the infected cells smaller than in the two *vivax* strains but the elongate forms occurred more frequently and were more striking in appearance. The parasitized cell was often considerably longer than wide (Plate 2, Fig. 12, 19, Plate 4, Fig. 29, Donaldson). Some of the cells were pear-shaped, with one end rounded and the other drawn out into one or more points (Plate 2, Fig. 18, Donaldson); in others the cell was pointed at both ends (Fig. 19); or the outline occasionally presented a series of ragged points or fimbriations of varying depth (Figures 12 and 17). These irregularities of cell outline occurred often enough to be considered a characteristic of the Donaldson strain.

About 20 per cent of infected cells containing all stages of both Chesson and St. Elizabeth strains were elliptical but only 2 per cent were definitely elongate, while in similar counts on the Donaldson strain, 35 per cent of all infected cells were ellipitcal and 16 per cent had a definitely long, narrow oval or otherwise

TABLE 3

Percentage of cells containing Schüffner's stippling with ring forms of the three strains

STRAIN	WITH 200 YOUNG RINGS EACH	WITH 200 OLDER RINGS EACH	TOTAL RINGS: 400 EACH STRAIN
Chesson	13	60	37
St. Elizabeth	20	63	41
Donaldson	29	90	60

elongated form. About 18 per cent of a sample of 300 binucleate schizonts of Donaldson were in extremely elongated cells.

In order to determine whether or not the percentage of cells containing Schüffner's stippling with ring forms of the Donaldson strain differed from the percentage showing Schüffner's with like stages of the two *vivax* strains, a count was made using only ring shapes without pseudopodia or early ameboid appearance. A conscientious attempt was made to separate the rings of each strain into (1) very young rings and (2) somewhat older rings. The results are presented in Table 3. It is apparent that when stained under identical conditions the rings of the Donaldson strain were more often accompanied by Schüffner's stippling than were those of the *vivax* strains.

With the staining technic used, all parasites beyond the ring stage in all three strains were accompanied by Schüffner's stippling. The granules became coarser, often more numerous and considerably more conspicuous as the parasite aged, being clearest and largest with the full grown parasite.

Among 2,500 infected erythrocytes containing various stages from each strain, taken from several patients in each instance, double infections of the cells were found in 3 per cent of the Donaldson, 4 per cent of the St. Elizabeth, and in 5 per cent of the Chesson strain cells. All stages from rings through mature schizonts and gametocytes were seen in combination in each strain, but by far the greater number of double or multiple infections consisted of ring stages.

The Donaldson strain, having rather compact appearing parasites, gives a quartan-like picture in the thick film except for the occurrence of Schüffner's stippling or the pink ghost cells around the parasites, which phenomena are characteristic of *vivax*.

DISCUSSION

The morphological characteristics of the Donaldson strain are much more like the published descriptions of *P. ovale* than they are like the two strains of *vivax* studied. A few of our observations do not tally with those of some workers on *ovale* but the workers in turn are not always in agreement on all characteristics.

The areas of the 100 rings in each of the three strains studied here are very similar, and individual rings could not have been identified as to species. The older Donaldson parasites and their containing erythrocytes are much smaller than those of either of the *vivax* strains studied and the enlargement of the cell

containing the mature schizont of Donaldson is only 27.4 per cent over the uninfected cell. These characteristics of the Donaldson strain appear to simulate those described for *P. ovale* by Stephens (1922).

The consensus of the literature on the appearance of the growing trophozoite of *ovale* is that it is solid and compact (quartan-like) and has few or no pseudopodia. In this respect, also, the Donaldson strain is like *ovale* and unlike *vivax*.

Although the majority of the Donaldson strain parasites were found in rounded erythrocytes, the number of infected cells that showed oval or elongate shape was considerably greater than it was in either of the known *vivax* strains. The *ovale* parasite gets its name from the oval shape of many of the infected erythrocytes, but Wilson and Wilson (1935) state that typical oval shapes are always in a minority in *ovale*, and Beet (1945) concurs. Fimbriation of outline of infected erythrocytes, generally considered a characteristic of *ovale*, occurs often enough in the Donaldson strain to be a good point of differentiation from the *vivax* strains.

Gametocytes seemed considerably less frequent in the Donaldson strain than in the two *vivax* strains. Boch (1939) speaks of the scarcity of gametocytes in *ovale*, while Wilson and Wilson (1935), on the other hand, speak of a larger output of "gametes" in *ovale* than in *vivax*.

Of 100 gametocytes measured in the Donaldson strain (65 female and 35 male gametocytes) 50 per cent were in slightly oval or truly elongate cells. Twenty-five per cent of the cells containing the gametocytes were more or less fimbriated. Wilson and Wilson say that *ovale* gametocytes are commonly in circular cells, and James, Nicol and Shute (1933) say that they are seldom or never contained in oval cells. These last two observations differ from our findings above for the Donaldson strain.

In lightly stained blood films the pigment of the Donaldson strain did not have any consistent outstanding features by which we could differentiate the strain from the two strains of *vivax* studied. In the literature on *ovale*, there is considerable difference of opinion in the description of shape, size, color, and arrangement of pigment granules.

With the very young ring forms, in all three strains, the Schüffner's stippling was very light, delicate and in such small granules as to be almost indistinguishable at times, even when the dots appeared large, dark and heavy with an older parasite in an adjacent cell. Often the Schüffner's granules with a young ring were quite scanty, at other times they seemed numerous. The 60 per cent of all Donaldson ring forms which were accompanied by Schüffner's stippling was considerably below the almost 100 per cent claimed for rings of *ovale* by James, Nicol and Shute (1933). The difference in staining must be the critical point here, for Stephens (1922) stated that the ring forms of *P. ovale* generally showed no Schüffner's stippling, though the dots were well marked in later stages; and Boch (1939) found Schüffner's stippling with *ovale* ring forms only occasionally even with intensive Giemsa staining, whereas with older forms it was even more noticeable than with *vivax*. Sforza (1948) said that Schüffner's dots became

coarser in more advanced stages of development of *ovale*, and Beet (1945) found an increase in the number of Schüffner's dots as the parasite became older. As has been stated, both of these observations apply to the Donaldson strain, also. In some instances in the Donaldson strain Schüffner's stippling outlined the infected cell but this was not so pronounced as found in *P. ovale* by James, Nicol and Shute (1933) with their method of staining. In all three strains studied, the Schüffner's granules were fairly uniform in size in any particular cell, though on occasions, larger, denser granules stood out prominently among the more uniform granules. This seemed slightly more prounounced in Donaldson than in the *vivax* strains.

When the thick film dries slowly, there may be in *vivax* infections compact forms that resemble Donaldson parasites; so, unless the examiner is quite familiar with the appearance of the Donaldson strain when compared to the *vivax* strains in the thick film, it is doubtful if the strains could be invariably differentiated by this method. With a stain that does not bring out Schüffner's stippling or resulting pink ghost cells around the parasite in the thick film, the Donaldson strain might be confused with *P. malariae*. James, Nicol and Shute (1933) make the statement that *P. ovale* cannot be identified in the thick film.

SUMMARY

A strain of malaria parasite (termed the Donaldson strain) recovered from a man who had served as an Army officer in the West Pacific during World War II, is compared morphologically with two known strains of *P. vivax*, St. Elizabeth and Chesson, to determine the taxonomic relationship of the strain. In the measurement of areas of three corresponding stages of each of the three strains and of the erythrocytes containing these stages, in the counts of the numbers of merozoites per mature schizont, in the observations on the shape of growing trophozoites and on the shapes of the parasitized erythrocytes, as well as on the thick-film appearance of the strain, the Donaldson strain differs greatly from the two *vivax* strains. In all of these respects it resembles published descriptions of *P. ovale*. There are minor differences between the strain and descriptions given by some of the workers on *P. ovale*. Some of these differences may be readily attributed to a difference in staining technic.

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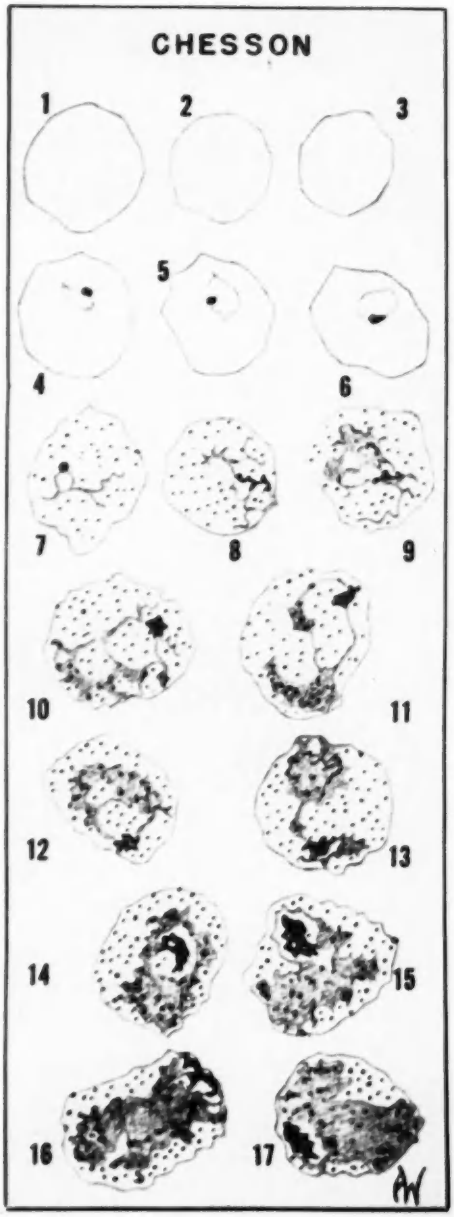
PLATE 1



PLATES* 1 AND 2

Polychromatophilic erythrocytes—No. 1‡ of each strain (Chesson, St. Elizabeth, and Donaldson).

Other normal erythrocytes—No. 2 and 3 of each strain.

Ring stages—No. 4, 5, and 6 of each strain.

Growing trophozoites—Chesson, No. 7–15; St. Elizabeth, No. 7–14; Donaldson, No. 7–19.

Mature trophozoite—Chesson, No 16 and 17; St. Elizabeth, No. 15 and 16; Donaldson, No. 20.

* Camera lucida drawings, all magnifications 1900 ×.

‡ Numbers refer to the numbers under the respective strain name in each of the four plates.

PLATE 2

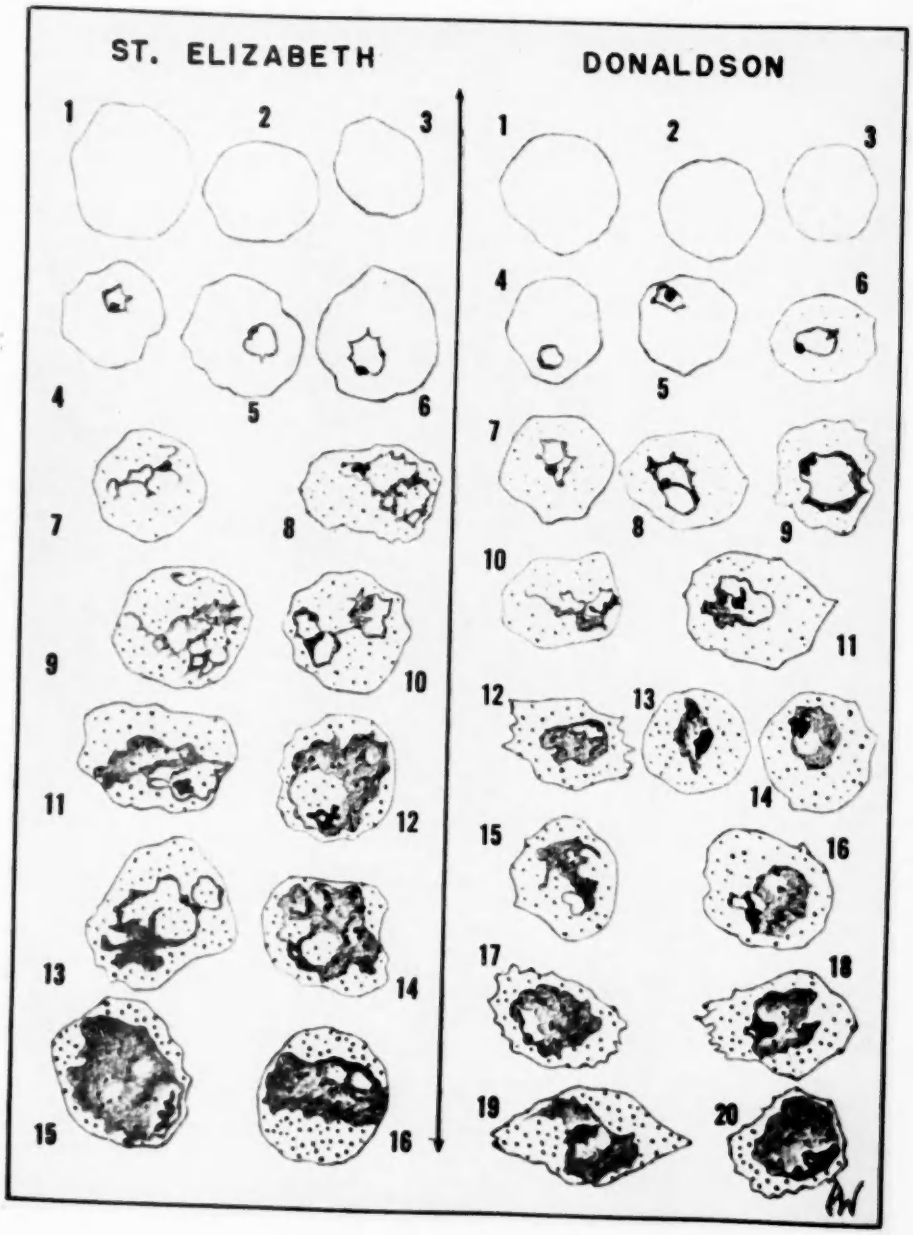

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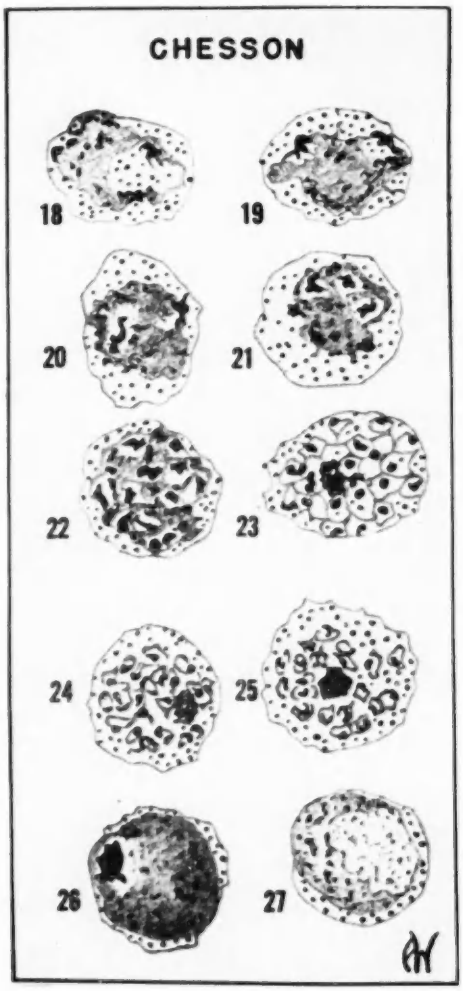
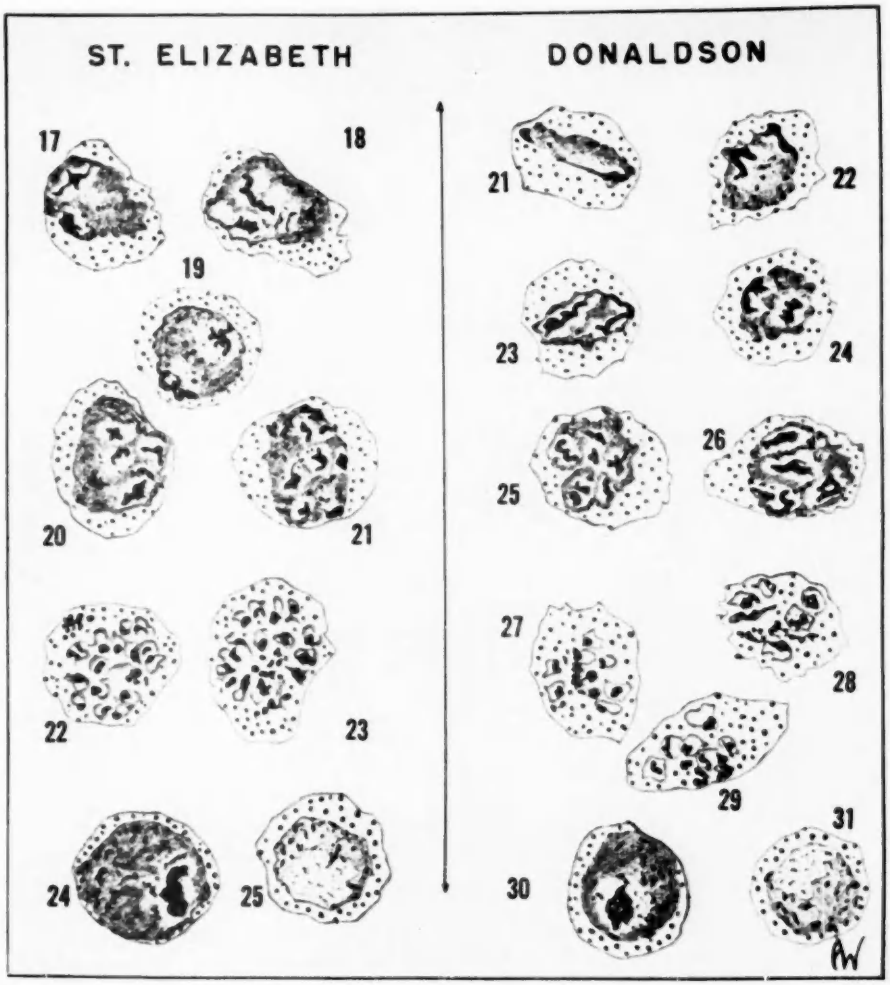
PLATE 3



Immature schizont—Chesson, No. 18-23. Mature schizont—Chesson, No. 24 and 25. Macrogametocyte—No. 26. Microgametocyte—No. 27.

PLATE 4



Immature schizonts—St. Elizabeth, No. 17–21; Donaldson, No. 21–26. Mature schizonts—St. Elizabeth, No. 22 and 23; Donaldson, No. 27, 28, 29. Macrogametocyte—St. Elizabeth, No. 24; Donaldson, No. 30. Microgametocyte—St. Elizabeth, No. 25; Donaldson, No. 31.

THE DONALDSON STRAIN OF MALARIA

3. The Infection in the Mosquito

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Two previous reports (Jeffery *et al.* and Wilcox *et al.* 1954) have described the morphology and infection characteristics of the Donaldson strain, pointing out its similarity to *Plasmodium ovale*. The present report will describe the characteristics of the strain in the insect host, and will demonstrate further its variance with *P. vivax*.

METHODS

The methods for observing the course of infection in the human host have been described previously (Jeffery *et al.*, 1954). For the study of the infection in mosquitoes three strains of *Anopheles* were used: *A. quadrimaculatus* and two strains of *A. albimanus* (the Panama and Florida Keys strains). The general procedures consisted of feeding lots of about 100 female mosquitoes on the carrier at the desired time; these were maintained after feeding in an insectary at 74–79°F. and were dissected and the oocysts counted 11 to 13 days, usually 12, after the blood meal. An average of 24.2 mosquitoes per lot was dissected. Where information on the comparative susceptibility of the three anophelines to the infection was desired, lots from all three strains were fed simultaneously so that comparison was direct.

The studies on the oocyst pigment arrangement necessitated dissection of specimens 4 to 10 days after feeding. For this study oocysts of two strains of *P. vivax*, the Chesson strain and a Korean strain, were studied for comparison with those of the Donaldson strain.

In most lots of mosquitoes fed, specimens which remained after routine dissection were maintained for use in transmission or for determination of the length of the extrinsic incubation period.

In a number of cases feedings were done daily or every other day in an effort to determine a pattern of infectivity over the entire primary course of infection in the patients.

OBSERVATIONS

Infectivity to A. quadrimaculatus *and* A. albimanus. Both of these species were susceptible to infection, and the comparative susceptibility of *A. quadrimaculatus* and the two strains of *A. albimanus* was determined. Wide variations in the relative susceptibility appeared frequently in different feedings. Occasionally one anopheline strain showed a much greater susceptibility than the others, while in other feedings the reverse would be true. The uncertainty and unpredictability of mosquito infections in the Donaldson strain necessitated a total of 120 com-

TABLE 1

Reliability of the results of 36 feedings comparing the susceptibility of A. quadrimaculatus *(Q-1) and the Panama (A-2) and Florida Keys (A-3) strains of* A. albimanus *when considered as paired lots*

STRAIN	NO. DISS.	MEAN % INF. WITH S.E.	P-VALUE BETWEEN SPECIES	MEAN OOCYSTS PER GUT	RATIO OF MEAN OOCYSTS	AVER. RATIO OF MEAN OOCYSTS NUMBERS WITH S.E.*
Q-1	1088	53 ± 5.1	0.332	15.0	1.1	1.6 ± 0.18 (P = .00046)§
A-3	1025	46 ± 5.1		13.4		
Q-1	1088	53 ± 5.1	0.003†	15.0	1.4	2.1 ± 0.37 (P = .00198)§
A-2	1077	33 ± 4.4		10.8		
A-3	1025	46 ± 5.1	0.052‡	13.4	1.2	1.3 ± 0.11 (P = .00634)§
A-2	1077	33 ± 4.4		10.8		

* Average of individual ratios in each pair of lots fed.
† Susceptibility greater in Q-1.
‡ Susceptibility greater in A-3.
§ Probability of difference from hypothetical 1-ratio.

parative feedings in order to accumulate a total of 36 suitable for good comparison of infection rates and intensities. Table 1 summarizes these observations in 36 comparative feedings and presents the reliability of the comparative susceptibility figures.

The percentage of mosquitoes infected was highest in *A. quadrimaculatus* and lowest in the Panama strain of *A. albimanus*. The superiority of *A. quadrimaculatus* over the Panama *A. albimanus* was statistically significant (P = 0.003); in comparing *quadrimaculatus* with the Florida Keys *albimanus* the difference in percentage infected was not significant (P = 0.332). The Florida Keys strain was probably significantly superior in susceptibility to the Panama *albimanus* (P = 0.052).

The intensity of infection as measured by oocyst numbers was also highest in *A. quadrimaculatus* and lowest in the Panama strain of *A. albimanus*. Mean ratios were determined by averaging the ratios of individual paired lots, varying from a ratio of 2.1 in the Q-1/A-2 comparison to 1.3 in the A-3/A-2 comparison. In all pairings the ratios of mean oocyst numbers were significantly higher than the hypothetical 1-ratio which should have been observed had the susceptibility of the mosquito strains been the same (P = < 0.01 in all three pairings).

Considering both infection rate and mean oocysts, the relative susceptibility might be expressed as follows: *A. quadrimaculatus*, 100; *A. albimanus* (Florida Keys), 77; *A. albimanus* (Panama), 46.

Infectivity to mosquitoes in relation to day and density of erythrocytic parasites. Because of the apparent uncertainty and inconsistency of infectivity, an effort was made to determine at what point during the infection conditions were most favorable for mosquito infection. In spite of numerous serial feedings throughout the course of infection in a large number of patients no stable pattern of infectivity has emerged. Mosquitoes were infected as early as the third parasite day

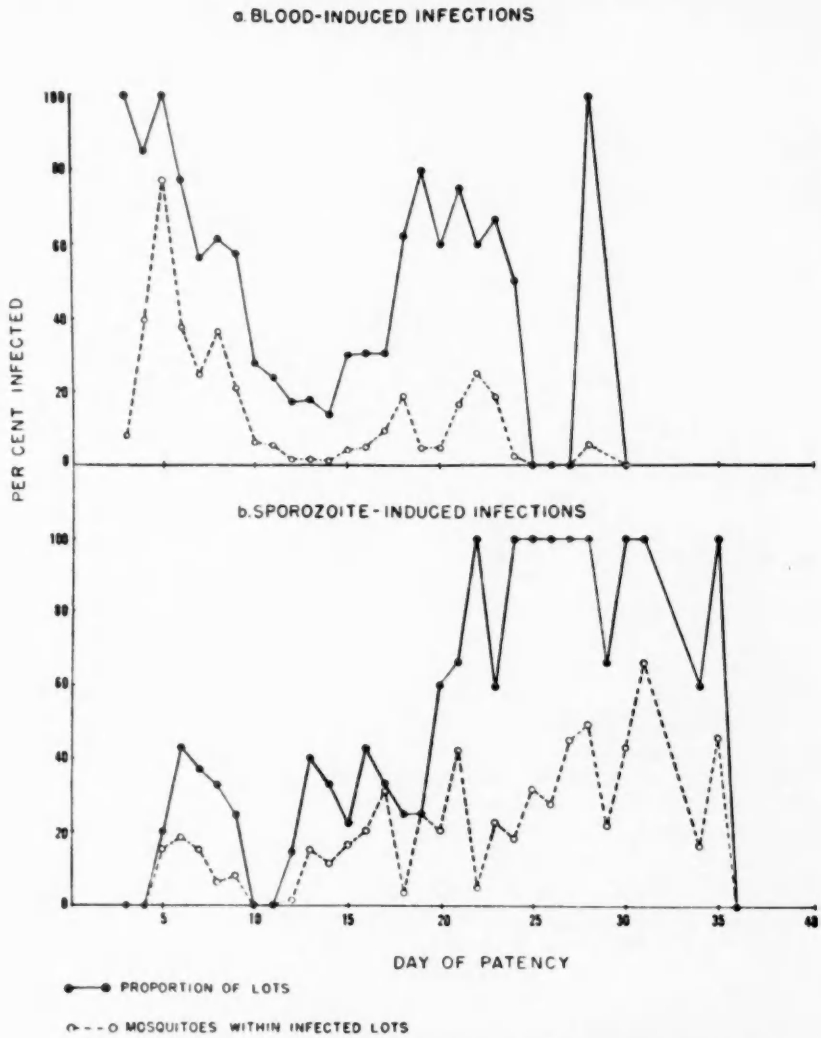

FIG. 1. Infectivity to mosquitoes of 42 blood-induced and 20 sporozoite-induced infections in relation to day of parasite patency: Proportion of lots infected and mean rates of infection of individual mosquitoes.

and as late as day 35. In 27 primary attacks where adequate serial feedings (7 to 22) were done, 11 produced no infections in mosquitoes, or light infections on only a few days; 16 cases produced infected mosquitoes on 25 to 100 per cent of the feeding days, with only eight cases infecting on one-half or more of the feeding

TABLE 2

Infectivity to mosquito lots and individuals in relation to parasite days in sporozoite- and blood-induced infections of the Donaldson strain

Mosquito-inoculated—20 cases

	DAY OF PRIMARY ATTACK											
	3–4		5–9		10–12		13–19		20–36*		Total	
	Lots	Indiv.	Lots	Indiv.	Lots	Indiv.	Lots	Indiv.	Lots	Indiv.	Lots	Indiv.
Diss.	4	101	44	1071	23	566	46	1065	34	722	151	3525
Inf.	0	0	14	99	1	3	15	145	26	169	56	416
% Inf.	0	0	31.8	9.2	4.3	0.5	32.6	13.6	76.5	23.4	37.1	11.8

Blood-inoculated—42 cases

	DAY OF PRIMARY ATTACK									
	3–9		10–17		18–24		25–30		Total	
	Lots	Indiv.	Lots	Indiv.	Lots	Indiv.	Lots	Indiv.	Lots	Indiv.
Diss.	81	1776	113	2774	32	684	8	201	234	5435
Inf.	55	543	26	135	21	74	2	3	104	755
% Inf.	67.9	30.6	23.0	4.9	65.6	10.8	25.0	1.5	44.4	13.9

* Two further feedings in days 55 and 57 were negative.

days. In only one case (9 feedings) were mosquitoes infected on each feeding day.

In attempting to derive some sort of infecting pattern all mosquito feedings done on 62 cases were compiled with reference to the day of parasites. Figure 1 charts the infectivity to mosquitoes from days 3 to 37 of blood- and sporozoite-induced cases. From this compilation, two rather indefinite patterns emerged, one for sporozoite-induced and one for blood-induced infections. These patterns in general indicated alternating periods of high and low infectivity. Table 2 summarizes these patterns, showing the proportion of mosquito lots and individuals infected during the successive periods of higher and lower infectivity. The days are grouped artificially and on the basis of the infectivity levels.

In the 20 mosquito-induced cases, it appears that there is an early period of noninfectivity through day 4, followed by a period (days 5 to 9) of fair infectivity. Days 10 to 12 again were quite unproductive of infected mosquitoes, with days 13 to 19 showing fair infectivity. The highest infectivity appeared during the 20 to 35 day period. In the 42 blood-induced infections, the infectivity appeared to be good during the 3 to 9 day period, poor from 10 to 17 days, good from 18 to 24 days and poor during the later days (25 to 30). From the totals it would appear that the blood-induced cases are slightly more infective to mosquitoes than are the sporozoite-induced cases.

Table 3 attempts to correlate the total parasite densities with infectivity to mosquitoes. It can be seen that mosquito infection occurred over a very wide range of parasite densities, and that high densities did not guarantee mosquito infection.

TABLE 3

Relation between total parasite densities in the carrier and resultant infections in mosquitoes

PARASITES PER CMM.	LOTS DISSECTED			INDIVIDUAL MOSQ. FROM INFECTED LOTS		
	Total	No. Inf.	% Inf.	No. diss.	% Inf.	Av. oocysts*
0–500	68	30	44.1	647	26.2	2.0
501–1000	44	25	56.8	528	33.8	3.8
1001–2000	58	27	46.6	554	44.7	7.2
2001–4000	81	30	37.0	634	42.3	4.2
4001–8000	96	36	37.9	835	47.6	16.5
8001–16000	39	13	33.3	322	20.1	3.8
16001–32000	6	2	33.3	47	4.3	39.0
Total	392	163	41.6	3567	37.4	7.3

* Per infected gut.

The correlation of mosquito infectivity with gametocyte densities is rendered quite difficult by the uncertainty of gametocyte diagnosis. The female gametocytes may be easily confused with large trophozoites, and even the males are at times confused with asexual forms. The strain is characterized by a dearth of gametocytes, when compared with the strains of *P. vivax* with which we are familiar. No correlation could be seen between the female gametocyte densities and resulting infections in mosquitoes. The density of male gametocytes is undoubtedly more certainly estimated than the female or total gametocytemias and Table 4 shows their density in relation to the infectivity to mosquitoes.

It is apparent that there is a positive correlation between the density of male gametocytes and infectivity to mosquitoes. However, the difference between infection rates at low and high densities is not as great as might be expected on the basis of proportional increase of gametocyte densities and infection rates.

Lenth of the cycle in the mosquito. At a mean temperature of incubation of about

TABLE 4

Relation between male gametocyte densities in the carrier and resultant infections in mosquitoes

MALES PER CMM.	LOTS DISSECTED			INDIVIDUAL MOSQ. FROM INFECTED LOTS		
	Total	No. Inf.	% Inf.	No. Diss.	% Inf.	Av. Oocysts*
0–25	235	75	31.9	1546	30.4	3.6
26–50	102	50	49.0	1094	42.9	8.2
51–100	38	23	60.5	544	42.6	9 0
101–200	16	14	87.5	359	44.4	16.1
201–400	1	1	100.0	24	62.5	86.2
Total	392	163	41.6	3567	37.4	7.3

* Per infected gut.

TABLE 5

X-pattern of pigment in oocysts of Donaldson strain and two strains of P. vivax

STRAIN	NO. OF OOCYSTS	NO. WITH X-PATTERN	PERCENT WITH X-PATTERN
Donaldson	206	70	38.2
Chesson *P. vivax*	323	26	8.0
Korean *P. vivax*	194	14	7.2

77°F. (range 74–79) the cycle is complete in an average of 15 days. Sporozoites have been found in the glands as early as 14 days and have been delayed to as late as 18 days, but they usually first appear in 15 days.

Arrangement of pigment in the oocyst. In establishing the identity of the Donaldson strain it was thought desirable to investigate the arrangement of pigment in the oocysts. For comparison the oocyst pigment in two strains of *P. vivax* was also studied (the Chesson strain and a Korean strain). Because of the similarity in other respects of the Donaldson strain to *P. ovale* the reportedly typical X-shaped figure was investigated. The results of these studies are given in Table 5.

It is apparent that the incidence of the X-shaped figure is much higher in the Donaldson strain (38.2 per cent) than in the Chesson (8.0 per cent) or Korean strains (7.2 per cent). The cysts examined ranged from 4 to 10 days old, and the X-figure was found only rarely in cysts as young as 4 days. Elimination of this 4-day group of 52 oocysts results in an incidence of 42.8 per cent in the Donaldson strain. Although there is a significantly greater incidence in the Donaldson strain, it is certain that this X-pattern does occur in the strains of *P. vivax*, and that it is present in well under half of the Donaldson strain oocysts. It is quite apparent that the oocyst pigment pattern varies as the cyst is rotated. Thus it is difficult to determine at which plane one is seeing the "normal" pigment pattern.

In general the Donaldson strain pigment granules differ from those of *P. vivax*. They are in smaller numbers, more irregular in shape and somewhat darker in color. In the Donaldson strain the granules occur almost invariably in a linear arrangement, while in *P. vivax* the granules are frequently in an amorphous arrangement, but may also be seen in a linear pattern.

DISCUSSION

The ability of the Donaldson strain to infect *A. albimanus*, apparently a non-indigenous mosquito, is in contrast to results reported for many strains of *P. vivax*. Boyd *et al.* (1938) reported that *A. albimanus* from Cuba and Panama showed high susceptibility to infection by coindigenous strains of *vivax* but were refractory to Nearctic strains. However, where coindigenous strains of *vivax* were concerned, *A. quadrimaculatus* was superior in susceptibility. Young *et al.* (1946) found *A. albimanus* almost completely refractory to infection by two New Guinea strains of *P. vivax*, although *A. quadrimaculatus* was very susceptible. Unpublished work done in our laboratories indicates that the same is true of a United States strain, a New Guinea strain and several Korean strains of *P.*

vivax. Although the Panama strain of *albimanus* was significantly inferior to *A. quadrimaculatus* in susceptibility to the Donaldson strain, the relatively high susceptibility of both *albimanus* strains is in contrast to their usual insusceptibility to many nonindigenous strains of *vivax* and is suggestive of a specific difference.

Few references have been found in the literature on comparative susceptibility of various mosquitoes to *P. ovale*. Apparently the only widely used mosquito host is *A. atroparvus*, although it has been suggested that, because of its geographic distribution, *P. ovale* must develop in numerous species of anophelines (Brumpt, 1949 in Boyd, Malariology). Lisova *et al.* (1946) noted successful infection of *A. sacharovi*, *A. superpictus*, *A. pulcherrimus* and *A. bifurcatus*, with the per cent of infection in *sacharovi* being 39 and in *superpictus* 29. However, there is some doubt as to the specific status of the malaria strain used.

Analysis of the data on infectivity of the Donaldson strain to mosquitoes in relation to day of parasites yielded little of significance. It can be seen (Table 2) that there may be waves of infectivity in both blood- and mosquito-inoculated cases, with the blood-inoculated showing a more regular pattern. Perhaps the most interesting observation was the higher infectivity occurring late in the course of the sporozoite-induced cases (20 to 36 day period). Most striking was the general low infectivity of the strain. Even during the period where the greatest proportion of lots contained infected mosquitoes the percentage of specimens infected averaged only 23.4 for mosquito-induced and 30.6 for blood-induced infections. In this respect the Donaldson strain is similar to *P. ovale*, with which James *et al.* (1932) obtained an infection rate of 54 per cent in *A. atroparvus*, but always found very weak infections in terms of oocyst densities, even though the batch of mosquitoes had been fed on the carrier on five successive days.

There appeared to be no orderly relationship between total parasite densities in the carrier and resultant infections in the mosquitoes. As might be expected the gametocyte densities showed some influence on the infectivity to mosquitoes, but even in this respect the results were not too well-defined. Probably due to the greater certainty of identification, the density of male gametocytes seemed to exert more influence over infectivity than did the female gametocytes. It is perhaps significant that about 30 per cent of the mosquito lots became infected when fed on male gametocytemias of 0 to 25 per cmm. The proportional increase of infectivity with gametocyte numbers was reflected much more emphatically in the number of lots infected than in the quality of infection within these infected lots.

P. ovale is generally considered as evidencing comparatively low numbers of gametocytes (James *et al*, 1932), although no references could be found to correlation of these densities with infectivity to mosquitoes. James *et al.* (1932) succeeded in infecting one mosquito out of 22 fed when the male gametocyte density was 12 per cmm. of blood.

The 15-day extrinsic incubation period in the Donaldson strain seems to conform very closely to that reported for *P. ovale*, and exceeds that reported for most strains of *P. vivax*. James, Nicol and Shute (1932) reported completion of develop-

ment of *ovale* in 16 days in *A. atroparvus* at 77°F. Sinton, Hutton and Shute (1939) using different strains noted complete development in 15 days, whereas under similar condition *P. vivax* required 10 and *P. falciparum* 12 days. In this laboratory the Chesson strain of *P. vivax* develops in *A. quadrimaculatus* in about 12 days and a Panama strain of *P. falciparum* in 14 days when incubated under the same conditions as the Donaldson strain.

The study of the pigment in the oocysts of the Donaldson strain indicate that it very closely resembles that of *P. ovale*. The recent study of Shute and Maryon (1952) has somewhat clarified the incidence of the reportedly typical X-pattern of the pigment in *P. ovale* oocysts. Most of the previous literature would indicate that this pattern is very nearly an invariable characteristic, while Shute and Maryon modify this to "a tendency to form chains which cross each other at right angles," and "often crossing at right angles". Further, in their plate showing the pigment pattern of *P. ovale* in 109 oocysts, only 36, or 33 per cent have an obvious X-pattern. Even in the oocysts between 6 and 10 days old, presumably the period of best pigment organization, the incidence of the X-pattern in apparently under 50 per cent. These figures compare favorably with the incidence of the X-pattern (38.2 per cent) that we found in the Donaldson strain.

Our observations on the Donaldson strain agree with those of Shute and Maryon on *P. ovale* in the linear arrangement of the pigment granules, the smaller number of grains, and the differing in shape from those of *P. vivax*. Our observations on the arrangement of pigment in *P. vivax* also seem to be in agreement with these workers.

SUMMARY AND CONCLUSIONS

The Donaldson strain of malaria was infective to *Anopheles quadrimaculatus* and to two strains of *A. albimanus*. *A. quadrimaculatus* showed the greatest susceptibility and the Panama strain of *albimanus* the least.

No close correlation could be observed between the day of parasite patency and infectivity to mosquitoes. There was some indication that there may be alternating waves of good and poor infectivity.

There was no orderly relationship between total parasite densities and infectivity to mosquitoes. The infectivity could be positively correlated with the male gametocyte density.

Gametocyte densities in the carrier were generally low, and infectivity to mosquitoes of much less certainty and intensity than with strains of *P. vivax*.

The extrinsic incubation period required about 15 days at 77°F.

The pigment granules in the oocyst occurred almost invariably in a linear arrangement, often with two lines crossing to produce an X-pattern. The granules were smaller in number and more irregular in shape than *P. vivax*.

Similarities between the Donaldson strain and *P. ovale* are discussed.

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THE DONALDSON STRAIN OF MALARIA

4. An Evaluation and Status

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The preceding three papers in this series have dealt in detail with the history and morphology of this strain and the characteristics of the human and mosquito infection (Jeffery *et al.* 1954; Wilcox *et al.*, 1954). The object of the present report is to evaluate the data and to arrive at some conclusion as to the specific status of this parasite.

This parasite has been under intensive observation for the past four years and has been passed through more than 150 patients. In addition many thousands of mosquitoes have been infected. At the same time known strains of *vivax*, *malariae*, and *falciparum* malaria were maintained in the laboratories so that it has been possible to compare all phases of these strains with the Donaldson strain. It had been hoped that a strain of *Plasmodium ovale* originating from an indigenous area might be carried simultaneously in the laboratory, but none has been available. For comparable *P. ovale* data, it has generally been necessary to use those quoted in the literature. In some instances it was possible to use stained blood smears containing these parasites. These were obtained from Mr. P. G. Shute at the Horton Mental Hospital, who was able for some years to maintain *Plasmodium ovale* in patients.

COMPARISON OF THE DONALDSON STRAIN WITH TWO STRAINS OF *P. vivax* AND WITH *P. ovale*

The various characteristics of the parasite strain which we have been able to measure are consolidated in Table 1, with comparable data for two strains of *P. vivax*. Such data for *P. ovale* as we have been able to secure from the literature on these comparative points are also listed in this tabulation.

Considering first the erythrocyte infected by the four types of parasites under discussion, it is obvious that the *vivax* parasites cause the red cells to enlarge more than do the Donaldson or the *ovale* types. Furthermore, there is an observable difference in the shape of the infected erythrocyte, the *ovale* shape and fimbriation being more common in the Donaldson strain than in the two *vivax* strains.

There was also a consistency in the shape of the parasite itself, in that the *vivax* parasite tended to be more ameboid as compared to a rather consistent compactness apparent in the Donaldson and the *ovale* strains. In this compactness the Donaldson and *ovale* strains resemble very closely *Plasmodium malariae*.

By a system of measuring the areas occupied by the parasites (Wilcox *et al.*, 1954) it is very definitely evident that the Donaldson parasite is smaller than either of the two *vivax* parasites under consideration, showing similar measurements to those of *ovale* parasites from the slides that we had obtained from Mr. Shute.

TABLE 1

Characteristics of the Donaldson strain as compared to vivax *and* ovale

CHARACTERISTIC	P. VIVAX STRAINS		DONALDSON	OVALE
	Chesson*	St. Eliz.†		
Infected RBC				
Enlargement/normal (ratio)	1.4	1.37	1.18	1.1
Oval shape (definitely elongate)	2%	2%	16%	Frequent
Fimbriation	Rare	Rare	Frequent	Frequent
Parasite shape	Ameboid	Ameboid	Compact	Compact
Parasite area (mean sq. microns)				
Ring	49.3	49.5	49.3	—
Binucleate schizont	75.4	71.3	62.5	—
Mature schizont	76.8	74.6	62.8	62.7‡
No. of merozoites—mean	17.3	14.0	7.8	8
Schuffners stippling				
Ring stage	37%	41%	60%	Frequent
Other	100%	100%	100%	100%
Infectivity to Negroes	Rare	Rare	Consistent	Consistent
Prepatent period—median days	12	14	15	15
Incubation period—median days	13	15	17	11–16
No. paroxysms—median	13	21	8	Less than 10
Max. parasites per cmm.—mean	39,890	15,506	8,314	Lower than *vivax*
Max. fever—mean°F.	105.3	106.0	105.2	—
Periodicity—hours	44.6	43.4	49.5	48 (?)
Response to drug	Moderate	Moderate	Very rapid	Very rapid
Relapse after RX—(chlor)	Frequent	Frequent	Rare	Rare
Immunity to:				
Chesson	High	None	None	
St. Elizabeth	None	High	None	
Donaldson	None	None	High	
Falciparum	None	None	None	None
Mosquito susceptibility‖				
A. quad to	++++	++++	++	
A. albimanus to	±	±	++	
Extrinsic Incubation at 77°F., days	12	12	15	15
Usual oocyst pigment pattern	Amorphous or double line	Amorphous or double line	Linear	Linear
Per cent X-pattern	8	7§	38	33

* South Pacific (tropical zone) strain
† U. S. A. (temperate zone) strain
‡ Measurement done locally on slides furnished by Mr. P. G. Shute.
§ Observations on Korean *P. vivax*, also a temperate zone strain.
‖ Plus system indicates approximate relative susceptibility.

One of the most striking differences in the morphology of the parasites was in the number of merozoites found in mature segmenters. The Donaldson strain averaged about half as many merozoites as either of the *vivax* strains and actually averaged fewer than the literature shows for *ovale*. With respect to merozoite number the Donaldson parasite resembles *P. malariae*.

The Schüffner's stippling was much more evident in the Donaldson-infected erythrocyte than in the *vivax*-infected erythrocyte. Here again the Donaldson strain seemed to agree with published reports of *ovale*.

When these strains of malaria under study were inoculated into human beings, the results also furnished points of comparison. Most of the white patients were susceptible to the two strains of *vivax* and to the Donaldson strain. However, the comparative susceptibility of Negroes to these three strains brought out a very striking difference. It has been shown by many workers and has very been evident in our laboratory for the past twenty years that the ordinary *vivax* malaria seldom infects Negroes. Occasionally Negroes will develop infections but most of these are aborted. In the case of Donaldson malaria, Negro patients consistently became infected, showing a normal primary attack; this characteristic has also been reported for *P. ovale*.

In the matter of prepatent and incubation periods, the Donaldson strain usually took longer to develop after inoculation by mosquitoes than did either of the *vivax* strains. The length of the primary clinical course of malaria offered another striking contrast as the Donaldson infection produced a much shorter primary course than did *P. vivax*. A similar contrast is found in the height of the parasitemia where again the Donaldson strain consistently produced fewer parasites. As to the mean maximum fevers produced by the various parasites, there was little difference between the *vivax* strains and the Donaldson.

The lengths of the asexual cycle of the parasites were different, those of the *vivax* being definitely shorter than 48 hours in length while the Donaldson strain exhibited a tertian periodicity of fevers with an average interval of about 49.5 hours.

The response to drugs offered another point of marked contrast in that the *vivax* infections usually gave a moderate response to the drug whereas the Donaldson strain infection was quickly eliminated, giving an exceedingly rapid response to almost any type of antimalarial drug. In this respect it agrees exactly with published reports of *P. ovale*.

The *vivax* strains are characterized by consistent relapses after the administration of so-called noncurative drugs such as quinine, quinacrine and chloraquine, whereas the Donaldson strain very rarely shows relapses after the giving of any drug or after spontaneous termination of the infection.

The Donaldson strain can be described as producing a mild infection in man because of its shorter primary attack, the lower maximum parasite densities, and the tendency not to relapse. The longer incubation period in mosquitoes might also be added to this evidence of being less virulent than the *vivax* infections.

There appeared to be no cross-immunity between the *vivax* strains and the Donaldson strain in any order of precedence. Nor could any cross-immunity be shown between the Donaldson strain and *P. falciparum* or *P.*[1] *malariae*.

When the host-parasite relationships of the Donaldson strain to the mosquito vector were studied it was determined that the Donaldson strain is unpredictable in its ability to produce infections in these insects. On occasions the strain was highly infective to both *Anopheles quadrimaculatus* and *A. albimanus*. It has been proved through exhaustive studies that the former species is highly susceptible to the Chesson and St. Elizabeth *vivax*, but that both of these strains of *vivax* show a very low infectivity to *A. albimanus*.

It took about three days longer for the Donaldson strain to mature in the mosquitoes than it did for the Chesson or St. Elizabeth strains, in which respect it again resembled *ovale*.

Some workers have described for *ovale* (James *et al.*, 1933) a characteristic pattern of pigment on the oocyst. We have found that the Donaldson strain oocysts also have this characteristic pattern of pigment arrangement. Although this pattern can be found in the two *vivax* strains studied by us, it was found four times as frequently in the Donaldson strain and might be used as an aid in separating the strains. On this particular point the Donaldson strain again resembled *ovale* more than it did the two *vivax* strains studied.

In virtually every point where comparison has been possible, the Donaldson strain differs from the *vivax* strains studied. In contrast, in every point where information was available, the Donaldson strain had characteristics similar to those described for *P. ovale*, and in some instances, such as the mean number of merozoites produced, it seemed to be even further removed from *vivax* than is *ovale*. These comparative data lead to the conclusion that the Donaldson strain of malaria actually is a strain of *P. ovale*.

EPIDEMIOLOGICAL SIGNIFICANCE

As far as can be determined, infections with *P. ovale* have never been established in this country. Craig (1914) was the first American worker to describe *ovale* in this country and he found it in a seaman who had just come from the Philippines. When he first saw the parasite he described it as a variety of *P. vivax* but some years later, after having seen *P. ovale*, he classified his parasite as *ovale*. During and following World War II there were several reports of possible *P. ovale* in blood smears taken from returning veterans. However, most of these turned out to be either normal or aberrant forms of *P. vivax* and to our knowledge there has never been any substantiated report indicating that a true *P. ovale* infection was diagnosed beyond doubt in troops from the Pacific area who had returned to U. S. A.

This leads us to believe that the present report of the Donaldson strain of *P. ovale* is the first documented record of such species being returned to this country by an infected person. It is significant that this infection was picked up either in the Admiralty Islands or the Philippines and that there have been previous reports of *P. ovale* being present in the Philippines (Craig, 1914). Malariologists will, of course, wonder how many infections of *P. ovale* were actually returned to the United States in the past ten years during the large scale movements of personnel to and from the Pacific areas. Although there is no way accurately to predict the number of such cases that might have returned, it appears unlikely

that the number would be high. Such factors as the reportedly low prevalence of the species in the areas concerned, the usual rapid response to drugs, and the infrequent relapses would serve to decrease the probability.

Nevertheless, our findings do point up the fact that *P. ovale* can be brought into the country even though precautions, such as the giving of suppressive drugs, have been taken. Although conditions now extant in this country appear to be unfavorable for the spread of exotic malaria, the fact that our native mosquitoes can be infected by this parasite and can transmit it to the human host indicates that this *ovale* type of parasite, which has never been established in North America, could actually become established under the right conditions. While the probability of such is not great, the possibility cannot be overlooked. The benign character of this type of malaria might well contribute to its establishment in this country or in others where suitable vectors are available. In a strain the symptoms of which are mild and in which spontaneous termination is the rule, detection and elimination of carriers would be difficult.

SUMMARY AND CONCLUSIONS

The Donaldson strain of malaria, which was recovered from a veteran returning from the Pacific area, has been intensively studied. All available evidence indicates that it belongs to the species, *Plasmodium ovale*.

This parasite is immunologically distinct from the native *vivax* malarias, it can be spread by native anophelines, and both Negro and white races are susceptible to infection. This indicates that this species which has never become established in the United States so far as known, could become established under favorable conditions.

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THE EFFECT OF PYRIMETHAMINE ON THE BI STRAIN OF *PLASMODIUM GALLINACEUM*

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Pyrimethamine (Daraprim), administered in a single dose of sufficient size and sufficiently early, prevented infections in chicks inoculated with sporozoites of the SP strain of *Plasmodium gallinaceum* (Greenberg *et al.*, 1953). Equivalent dosages of the drug, administered to chicks with an established parasitemia, prevented death from tissue forms, but did not eradicate the infection. It is possible that the pyrimethamine was able to eradicate all fixed tissue forms because death from these forms was prevented, but was not able to eliminate all the erythrocytic forms of the parasite. This possibility could be examined in our BI strain of *P. gallinaceum*. When this strain, which is usually transmitted by blood passage, is transmitted by mosquitoes, the resulting infection is overwhelmingly exoerythrocytic with no normal erythrocytic forms detectable microscopically or by subinoculation prior to the death of the chicks (Haas *et al.*, 1945 and 1948, Trembley *et al.*, 1951). If pyrimethamine were able to eradicate tissue forms, chicks with established sporozoite-induced BI strain *P. gallinaceum* infections could be cured. If they were not cured, but prevented from dying of tissue infections, we were interested in determining the nature of the persisting infection.

MATERIALS AND METHODS

Week-old New Hampshire red chicks from a single supplier and weighing from 45 to 55 grams were used as hosts throughout. The parasite was the BI strain of *Plasmodium gallinaceum*, the history and characteristics of which have been described (Haas *et al.*, 1948; Lewert 1948, 1950; Greenberg *et al.*, 1950; Trembley *et al.*, 1951; and Richeson *et al.*, 1953). Laboratory reared *Aedes aegypti* served as invertebrate hosts.

Inoculations of sporozoites by the one-mosquito equivalent method and of infected blood, as well as the preparation and reading of blood and brain smears, have been described in detail elsewhere (Coatney *et al.*, 1945 and 1953). Blood smears from sporozoite inoculated chicks were examined daily beginning on day 7 (day of inoculation is day 0) and continued to the twenty-third day. Blood smears from blood-inoculated chicks were examined daily beginning the day after inoculation and continued to the twentieth day. Brain smears were made of all chicks at autopsy. Pyrimethamine was administered orally by catheter in dilute aqueous lactic acid.

RESULTS

The results of administering graded single doses of pyrimethamine to chicks on the seventh day after inoculation of sporozoites are shown in Table 1. In

each series the untreated chicks died of an overwhelming tissue infection, with no normal erythrocytic parasites detected, between the ninth and eleventh day. The drug was effective in preventing death from tissue forms in all but five chicks at dosages of 0.0005 mg./gm. or more. However, none of the chicks were cured; all developed a parasitemia with normal, pigmented parasites. The day of appearance of normal erythrocytic parasites was roughly a function of the dose of drug administered, but the day of mean highest parasitemia was not. In all the chicks the parasitemia was of relatively low grade, and nearly all the chicks survived the peak parasitemia. When the surviving chicks were killed on the thirty-sixth day, only one exhibited tissue forms in brain smears. All the chicks which died, including one on the twenty-eighth day after inoculation, exhibited tissue forms in brain smears made at autopsy.

Series 1/5/53 was inoculated with sporozoites from mosquitoes fed on day 21 of series 12/2/52. The host chick had received pyrimethamine at 0.004 mg./gm. on day 7. The strain did not change its characteristic of producing only tissue infections in untreated chicks. Also, five additional lots of mosquitoes, fed on chicks of the 12/2/52 experiment (one lot fed on one chick from each of the dosage groups), developed sporozoites which produced only tissue infections in the recipient chicks. During two serial mosquito passages subsequent to 1/5/53, using chicks treated with pyrimethamine at 0.002 mg./gm. as donors, the strain characteristic did not change nor was there a change in response to the drug. None of the treated chicks died; none were cured; all developed normal parasitemia on the eighteenth to twentieth day. None were found to have tissue forms in the brain when they were killed on day 36. Mosquitoes fed on randomly selected donors 14 days after treatment became infected. All untreated chicks died of exoerythrocytic infections.

We then wanted to know when in the course of the infection in treated chicks pigmented parasites arose which were capable of multiplying in the erythrocytes. To answer this, five chicks were inoculated with sporozoites of the BI strain from mosquitoes fed on a blood-infected donor. On day 7 the chicks were treated with a single dose of pyrimethamine, 0.002 mg./gm. The course of the infection in these chicks (Table 2A) was comparable with those shown in Table 1. Blood smears were discontinued on day 22 and the chicks killed on day 36; no tissue forms were seen on examination of brain smears made at autopsy. Parasitemia was detected on the seventeenth to twentieth day. On days 11, 13, 15, 17, and 19, each of the chicks served as a donor for a clean chick into which 0.1 ml. of blood was inoculated through the jugular vein. The course of the infection was followed in the recipient chicks by daily blood smears, and brain smears of all the chicks were examined for tissue forms when they died or were killed.

A summary of the results of the subinoculations is shown in Table 2B. Two of the recipients of day 11 and one of day 13 failed to develop parasitemia; one of these chicks died of an overwhelming tissue infection. In general, the later in the course of a treated infection blood was subinoculated, the earlier the recipients exhibited parasitemia. This would indicate that the population of erythrocytic parasites was increasing in the donors but at submicroscopic levels. Tissue forms

were detected at autopsy in all but two of the infected recipients regardless of the day of subinoculation. There was no indication that tissue infections appeared later in the recipients, or were less severe as the infection progressed in the donors. It is apparent that the severity of the tissue infection, as determined by time of development and numbers found at autopsy, was similar in recipients from a single donor. Recipients from chicks 3606, 3607, and 3610 were heavily infected with tissue forms relatively early in the course of their infection; those from chicks 3608 and 3609 exhibited three types of infections, (1) no tissue parasites; (2) tissue parasites appeared early but were scarce; and (3) tissue parasites appeared only after a considerable time. In 14 of the recipients, tissue forms were present in sufficient numbers (3 or 4 plus) to be considered the sole or primary cause of death. These forms may also have caused the death of other chicks with apparently less heavy infections. Tissue forms were detected in 17 chicks which died before the sixteenth day after subinoculation. Most of the deaths from tissue forms occurred on the eleventh to the thirteenth day regardless of the previous course of the erythrocytic infection in the donor or the recipient.

DISCUSSION

The BI strain is capable of producing normal pigmented parasites and gametocytes and differs from the other strains of *P. gallinaceum* only in the length of time required for these forms to appear. In the SP and M strains erythrocytic forms are microscopically detectable 7 to 8 days after the inoculation of sporozoites; in the BI strain they are not microscopically detectable prior to the death of the untreated chick from exoerythrocytic parasites 10 to 11 days after inoculation of sporozoites. If death from tissue forms is prevented by the use of a suppressive drug, as we have done, or if the parasite is perpetuated by serial inoculations of infected blood, as was done by Lewert (1948, 1950, 1950a), and Haas *et al.* (1948), erythrocytic parasites appear. By the latter method erythrocytic forms were not found until the second passage or between 2 and 3 weeks after the original inoculation of tissue forms. This approximates the time required for these forms to appear in our experiments using pyrimethamine to keep the chicks alive. By subinoculation experiments we have found that few, if any, erythrocytic forms were present in chicks before the eleventh day after inoculation of sporozoites of the BI strain. It should be pointed out that correspondence between our present results and those of Haas *et al.* (1948) and Lewert (1950, 1950a), exists even though these investigators used exoerythrocytic tissue forms as their original inoculum and we used sporozoites. It should also be remembered that all three sets of results were accomplished *only* by artificial means, i.e. through the intervention of the investigators. Under normal circumstances the sporozoites or tissue forms of the BI strain do not produce erythrocytic parasites.

While the recrudescence of the infection in chicks inoculated with sporozoites of the BI strain and treated with pyrimethamine was predominantly erythrocytic, there is evidence that exoerythrocytic parasites were also being produced. This evidence was obtained by observing the course of the infection in chicks which

TABLE 1

The course of BI strain Plasmodium gallinaceum *infections in chicks treated with pyrimethamine 7 days after inoc lation. Five chicks were included at each dosage level*

DOSE OF PYRIMETHAMINE MG./GM. (day 7)	MEAN PARASITEMIA (PARASITIZED RBC/10^4 RBC) ON DAY								
	8	10	12	14	16	18	20	22	24
Series 12/2/52									
0.016	0	0	0	0	0	0	2	218	528[1]
0.008	0	0	0	0	0	2	6	166	1070[2]
0.004	0	0	0	0	2	18	296	138	132
0.002	0	0	0	6	6	4	308	1274	576
0.001	1	0	1	12	6	83	445	363	1413[3]
0.0005	0	0	5	10	14	92	1396	1916	689
0.00025	0	3	2	0	0	215	3440	2400	8[4]
None	Dead 10, 10, 10, 10, and 11; all ++++ tissue forms in brain smears.								
Series 1/5/53									
0.016	3	0	0	0	0	0	19	88	92
0.008	2	0	0	0	0	0	13	62	100[5]
0.004	1	0	0	0	0	1	70	892	824
0.002	0	0	0	0	0	4	258	1121	10
0.001	0	1	2	1	50	20	397	355	413[6]
0.0005	1	5	4	2	7	114	650	1438	24
0.00025	1	4	0	0	0	100	50	90	10[7]
None	All dead day 9, 10, 10, 10, and 11, ++ to ++++ tissue forms in brain smears.								

[1] One chick brain + on autopsy, day 36.
[2] One chick died day 26, brain +
[3] One chick died day 13, brain +
[4] Three chicks died day 13, brain +++
[5] One chick died day 28, brain ++
[6] One chick died day 14, brain ++++; one chick died day 18, brain ++++
[7] One chick died day 10, brain +++; five chicks died day 11, brain ++

TABLE 2A

The course of BI strain, Plasmodium gallinaceum *infection in chicks treated with pyrimethamine (0.002 mg./gm.) seven days after inoculation*

CHICK NO.	PARASITIZED RBC/10^4 RBC ON DAY												
	7	11*	12	13	14	15	16	17	18	19	20	21	36
3606	–	–	–	–	–	–	–	–	5	50			K[0]
3607	–	–	–	–	–	–	–	–	–	–	5	10	K[0]
3608	–	–	–	–	–	–	–	5	50	20	20	10	K[0]
3609	–	–	–	–	–	–	–	–	–	–	10	20	K[0]
3610	–	–	–	–	–	–	–	–	–	–	10	50	K[0]

* = Blood smears negative from day 7 to 11.
– = No parasites found.
K = Killed. Superscript [0] = No tissue forms found in brain smears.

TABLE 2B

Summary of infections in recipients subinoculated with 0.1 ml. of blood from chicks in Table 2A

SUBINOCULATED:			DAY OF:		TISSUE FORMS IN BRAIN SMEARS
From	To	On day†	First detectable parasitemia	Death	
	3611	11	None	*	0
	3616	13	8	15	++++
3606	3621	15	5	14	++++
	3626	17	4	13	++++
	3631	19	3	11	++++
	3612	11	None	12	++++
	3617	13	None	*	0
3607	3622	15	9	21	++++
	3627	17	7	14	++++
	3632	19	4	11	++++
	3613	11	9	18	++
	3618	13	8	12	0
3608	3623	15	6	10	++
	3628	17	4	9	+
	3633	19	1	7	0
	3614	11	9	20	++++
	3619	13	9	16	+
3609	3624	15	7	22	++++
	3629	17	4	9	++
	3634	19	3	17	++++
	3615	11	11	12	++
	3620	13	9	12	+++
3610	3625	15	7	12	++
	3630	17	5	11	++++
	3635	19	3	11	++++

† See Table 2A.
* Killed day 36.

received infected blood from the treated chicks (Table 2B). According to Haas *et al.* (1948) and our own experience (Greenberg and Coatney, 1953) death from exoerythrocytic parasites in chicks inoculated with erythrocytic parasites of the BI strain occurred between 16 and 24 days after inoculation. The presence of large numbers of exoerythrocytic parasites in many of the recipient chicks (Table 2B) 9 to 15 days after subinoculation and within a week of initially detected parasitemia must have arisen from exoerythrocytic precursors transferred from the donors. Such precursors were circulating in some of the donors as late as 19 days after inoculation of sporozites and 12 days after treatment with pyrimethamine. The number of such precursors in circulation appeared to vary from donor to donor and did not materially increase or decrease in any single donor from the eleventh to the nineteenth day.

If the data from Table 1, 2A, and 2B are pooled it becomes clear that the number of exoerythrocytic parasites present in chicks treated with pyrimethamine forms a continuum. Such forms were sufficiently abundant in some chicks (Table 1) to have contributed to their death; they were less common but still detectable on autopsy, 26 to 36 days after inoculation, in other chicks. In the remaining chicks exoerythrocytic forms were too rare to kill the chick or to be detected in routine examinations of brain smears. Their presence was proved by subinoculating blood into susceptible recipients. Even in the large group of chicks with no microscopically detectable exoerythrocytic forms, variation continued in the numbers of such forms present in the circulation. As judged by the results in the recipients, forms capable of invading the endothelium must have been more abundant in chick 3610 than in chick 3608 (Table 2B).

It seems evident from the data that pyrimethamine in the doses given does not eradicate the tissue stages of the parasite. Those parasites which escaped drug action continued to reproduce at variable, and usually reduced rates, thus conferring premunition on most of the chicks. This premunition would account for the relative immunity exhibited against acute relapses of exoerythrocytic infections following treatment with pyrimethamine.

SUMMARY

Pyrimethamine, in large single doses administered on the seventh day of a sporozoite-induced BI strain *Plasmodium gallinaceum* infection, did not cure the infection though it did prevent death from exoerythrocytic parasites. After a period in which there were no microscopically detectable parasites and which varied slightly with the dose of drug, normal pigmented parasites were found. Erythrocytic parasites could be demonstrated by subinoculation of blood into susceptible recipients as early as 11 to 13 days after inoculation. Variable and reduced numbers of exoerythrocytic parasites were found rarely on direct examination as late as 36 days after inoculation, and more frequently by subinoculation of blood at least as late as the nineteenth day. Mosquitoes could be infected when parasitemia was on the increase. The resulting sporozoites produced only exoerythrocytic infections in untreated recipients, indicating no essential change in the characteristics of the strain. The response of the parasite to the drug remained unchanged during four such serial transfers, using treated chicks as donors.

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RELATIONSHIP BETWEEN TIME OF ADMINISTRATION, DOSE, AND PROPHYLACTIC ACTIVITY OF PYRIMETHAMINE ON SPOROZOITE-INDUCED *PLASMODIUM GALLINACEUM* INFECTIONS

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Pyrimethamine has been shown to have prophylactic activity against *Plasmodium gallinaceum* infections in the chick (Falco *et al.*, 1951; Jaswant Singh *et al.*, 1952; Greenberg *et al.*, 1953). We have found that single doses of 0.004 mg./gm. body weight, administered 4 to 5 hours before inoculation of sporozoites, completely aborted the infection in most chicks. Doses as small as 0.0005 mg./gm., given under the same conditions, produced a significant delay in the appearance of parasites in the blood but most chicks eventually died of tissue infections. On the other hand, none of the chicks treated on day 7 with doses as high as 0.016 mg./gm. were cured, but doses as small as 0.0005 mg./gm. prevented death from tissue forms. These data seemed to indicate a fundamental change in the sensitivity of the parasite to the drug or in the immunological status of the host. The present report extends the original observations to determine when these changes occurred.

METHODS

New Hampshire Red chicks from a single supplier, weighing 45 to 60 grams, were used as hosts. The insect vector was *Aedes aegypti*. One mosquito equivalent dosage of sporozoites, according to the method described by Coatney *et al.*, 1953, was used in all the experiments. All doses of drug were given orally by catheter in dilute lactic acid. The method of inoculating blood, the making and interpreting smears of the blood and of the brain were as described by Coatney *et al.*, 1953. Beginning on day 7 (day of inoculation was day 0) smears of the blood were made and read daily for the first 21 days, then twice weekly until day 36 when all surviving birds were killed and smears were made from their brains.

RESULTS

The results of five independent series have been combined in Tables 1 and 2. In these series all but one of the 25 untreated chicks died of an overwhelming tissue infection.

As the dosage of pyrimethamine was increased, the number of chicks in which the infection was aborted (Table 1) increased from 1 out of 40 at 0.001 mg./gm. to 32 out of 40 at 0.016 mg./gm. Since the chicks cannot tolerate doses higher than the last one given, it is apparent that treatment could be withheld only until the sixth day if one expected to obtain cures. The increased cure rate with increased dose was mostly the result of withholding treatment during the time cures were

TABLE 1

The prophylactic effect of single doses of pyrimethamine administered on various days to chicks infected with sporozoites of P. gallinaceum

DOSAGE OF PYRIMETHAMINE (MG./GM.)	NO. CHICKS CURED*								
	Day of treatment								
	0	1	2	3	4	5	6	7	Totals
0.001	0(10)	1(10)	0(11)	0(12)	0(11)	0 (9)	0(10)	0(10)	1
0.002	3	5	1(12)	3	0(16)	0(13)	0(18)	0(16)	12
0.004	3	5	3	2(17)	0(14)	0(14)	0(17)	0(18)	13
0.008	4	5	2(15)	4	1(18)	1(19)	1(20)	0(18)	18
0.016	5	5	5	5	4	4	4	0(22)	32
Totals....	15	21	11	14	5	5	5	0	

* Five chicks were included at each dose on each day.

Figures in parentheses represent number of days before more than half the chicks exhibited parasitemia.

possible. Most of the chicks treated during the first three days were cured at doses of 0.004 mg./gm. Higher doses of drug were required to cure chicks with more advanced infections. A peculiarity of the results, which in spite of the small sample is probably real, was the greater number of cures obtained from treatment on day 1 than on any other day. It also required considerably less drug to effect 100 per cent cures on day 1 than on any other day. For 50 per cent cures, treatments on day 0 and day 1 were about equivalent, days 2 and 3 were comparable, and days 4, 5, and 6 were quite similar. It should also be noted that in those chicks not cured by a given dose there was no simple correlation between the appearance of parasitemia and time of treatment although there was a trend toward delay of parasitemia as the dose was increased.

TABLE 2

The prophylactic effect of single doses of pyrimethamine administered on various days to chicks infected with sporozoites of P. gallinaceum

DOSAGE OF PYRIMETHAMINE (MG./GM.)	NO. CHICKS WITH TISSUE FORMS*								
	Day of treatment								
	0	1	2	3	4	5	6	7	Totals
0.001	4	4	2	0	1	1	0	0	12
0.002	1	0	2	0	0	0	0	0	3
0.004	2	0	0	2	0	1	0	0	5
0.008	0	0	0	0	0	0	0	0	0
0.016	0	0	0	0	0	0	0	0	0
Totals.....	7	4	4	2	1	2	0	0	
Per cent of those not cured..	70	100	28	16	10	10	0	0	

* Five chicks were included at each dose on each day.

In Table 2 is shown the number of chicks which died of causes attributable to tissue forms. Again there was a dose response gradient; 12 of the 40 chicks treated with 0.001 mg./gm. died of tissue infections, while none of the 80 chicks treated with 0.008 or 0.016 mg./gm. died of this type of infection. The dose response gradient was chiefly the result of greater numbers of cures effected at higher doses, mainly among those chicks treated during the first two or three days. None of the chicks treated on days 6 or 7 died of tissue infection and only a sporadic few of those treated on days 4 or 5. If we consider the percentage of chicks not cured but which died of tissue infections, the highest percentages occurred on days 0 and 1, with an apparent gradual decline until on day 6 or 7 none of the chicks died of tissue infections.

DISCUSSION

The data in Table 1 can probably be explained as follows: as the infection progressed the numbers of parasites increased, so that the longer treatment was withheld the greater the challenge to the drug and the larger the dose required to eradicate the infection.

The material in Table 2 is somewhat more difficult to explain. It is apparent that the longer treatment was delayed the greater the chance for chicks with surviving infections not to die of tissue forms. It is possible that pyrimethamine affects late tissue parasites differently than early ones. However, it is apparent also that in terms of the minimal dose required to produce an effect, the late tissue parasite is no more sensitive than the early tissue forms of the parasite (Table 1, and Greenberg *et al.*, 1953).

It is quite likely that the results are a product of the interaction of host immunity and therapy, and that the immune status of the host was progressively changing. Untreated *P. gallinaceum* infections in the chick are usually fatal, regardless of the stage of the parasite inoculated. The infection is fulminating and the host usually dies before or shortly after a crisis, at which time numbers of parasites decline rapidly. When fewer parasites of any stage were inoculated (Greenberg *et al.*, 1950, 1953; Richeson *et al.*, 1953) survival time increased but not the survival rate. From this it can be concluded that immunity became apparent quite late in the course of an infection and that the length of time of association of parasite and host was not a controlling factor, but that total numbers of parasites might have been. However, in view of what happened when pyrimethamine suppressed the parasites, this conclusion would have to be revised. One could postulate that pyrimethamine-inhibited parasites provoked a greater immune response than a larger, more rapidly multiplying population of normal parasites. However, this would not explain the differences in effect produced by the drug when administered at various times after inoculation of sporozoites. Again numbers of parasites may have been critical, and the following explanation would appear to be a guide to further experiments: a certain number of parasites is required at the beginning of the infection in order to initiate an immune response. Once this response is started it progresses to a maximum regardless of the number or growth rate of the parasites. Normally the parasite increase

is greater than the immune response can handle until it reaches its peak efficiency, by which time the damage to the host is irreversible. If, however, treatment intervenes to control the numbers of parasites, *after* the immune response has been initiated, then peak efficiency of the response can be reached before overwhelming numbers of parasites have had an opportunity to develop. On the other hand, treatment *before* the immune response was initiated would leave the host unprotected after the effects of the treatment wore off. It is perhaps significant in this regard that Huff (1953) found evidence of damaged tissue forms of the parasite 53 hours after the inoculation of sporozoites.

SUMMARY

In studying the relationship between time of administration and dose of pyrimethamine against *Plasmodium gallinaceum* in young chicks, it was found that with single oral doses the infection could be eradicated even if treatment was withheld up to and including day 6. A greater percentage of chicks not cured by early treatment died of exoerythrocytic infection than of those not cured by treatment given later.

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STUDIES ON AVIAN MALARIA IN VECTORS AND HOSTS OF ENCEPHALITIS IN KERN COUNTY, CALIFORNIA

I. Infections in Avian Hosts[1, 2]

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In the more than 65 years since Danilewsky (1885) first reported finding erythrocytic parasites in birds, extensive experimental research has been conducted on this group of protozoa. The majority of these investigations have been surveys based on the examination of single smears of blood from wild birds or have been studies based on experimental procedures in the laboratory. There have been few extensive epizoological studies under natural conditions. When one considers the importance of Ross's (1898) discovery of mosquito transmission of malaria to avian hosts and the effect of this discovery on subsequent studies of human malaria, it is indeed surprising that up to the present the epizoology in nature of any avian *Plasmodium*, particularly with regard to the mosquito vectors, has not been studied comprehensively.

Hewitt (1940) listed the avian hosts of plasmodia reported up to 1940 and comprehensively reviewed the morphology and systematics of these parasites. The published host records of the blood protozoa of North American birds were reviewed by Herman (1944). Prior to this review, the blood parasites of birds of the Pacific Coast States of North America were surveyed by a number of investigators.* Subsequent to these reviews, several authors have searched for avian blood parasites in Washington (Wagner, 1946) and California (Herman, 1951). Coatney and Roudabush (1949) reviewed the world literature on *Plasmodium* from all hosts.

During studies of the mosquito vectors and avian hosts of the arthropod-borne viral encephalitides in Kern County, California, it became apparent that essential information pertinent to the epidemiology of these encephalitides could be gained by studying avian malaria. To acquire this information, a broad epi-

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* These reports are summarized by Herman (1944) and the references are not listed in the present paper unless cited elsewhere.

zoological investigation of avian malaria was undertaken in that vicinity in the summer of 1946 and was continued through the summer of 1951. This area was especially favorable for such a study because a great many of its characteristics had already been learned through the encephalitis studies of the last ten years. This six-year study yielded knowledge of the prevalent species of *Plasmodium*, the kind and proportion of birds infected, the mosquitoes that served as vectors and the relationships between *Plasmodium*, mosquito, and bird. In a broader sense, it was possible to study the ecology of avian malaria in a small well-known area rather completely in the hope of contributing to the epizoology of this disease. This report is concerned with the species, incidence and prevalence of *Plasmodium* infection in avian hosts. Subsequent papers will consider the mosquito vectors (Reeves, Herold *et al.* 1954; Rosen and Reeves, 1954) and epizoological correlations, including relationships to the viral encephalitides (Reeves, Herman *et al.*, 1954).

STUDY AREA

Kern County is in the southernmost part of the great Central Valley of California. The present studies were carried out in the level valley floor of the county, the valley being surrounded on three sides by mountain ranges. The elevation of the valley floor is 400 to 500 feet, and before the development of irrigation this area was largely desert, except for the Kern River bed and occasional intermittent streams. At present, this is one of the richest agricultural areas of the United States. During the summer the climate is hot and dry with an average daily mean temperature of 80°F.; in winter the mean temperature is 51°F. Rainfall is scant, less than six inches for each of the study years, but surface water is abundant because of irrigation.

Human malaria formerly was endemic in this area, and arthropod-borne viral encephalitides are endemic currently.

Three main study areas were selected for investigation: (1) six farmyards in the Shafter farming district with uniform plantings of low field crops, a few fruit, nut and shade trees in the yards, and usually a small reservoir for storage of irrigation water; (2) a 100-acre olive grove surrounded by a cultivated field-crop farming area; and (3) Hart Memorial Park, an irrigated wooded area of over 177 acres located on the Kern River and surrounded on all sides by natural uncultivated lands. Occasional studies were made in other domestic and rural areas.

In 1948, investigations were extended to include the city of Bakersfield. Birds were trapped in 9 locations: the premises of 7 private residences, a public park and the grounds of the county hospital. These areas differed from the others in that they were in a residential area where over 100,000 people live and where there was intensive mosquito control.

Twenty-one species of mosquitoes have been found in the county (Brookman and Reeves, 1952).

Bird populations and ecology of these vicinities were studied intensively and will be reported separately (McClure, 1952). In the six-year study over 200

species of birds were observed in the valley. The English sparrow and house finch were the most common resident species, and various species of migratory birds were quite common at certain seasons.

METHODS

Birds were collected in a variety of ways. Several types of traps were utilized, including various sizes of hardware-cloth funnel-traps and 8-cell figure-4 drop-gate traps. These were baited with natural bird foods. Collections were occasionally made by netting or shooting. Numerous nestlings were sampled from nests under observation.

A schedule of collecting was developed for the summer period to provide a sample of birds each week from the major study areas. Insofar as it was practical, the species and numbers sampled represented a cross section of the bird population currently predominating in each area. Some species of birds were not found in any of these major study areas; these were occasionally collected in adjacent outside areas and are included incidentally.

Age of the birds was recorded as: "nestling" for those in the nest, "immature" for birds, hatched during the year, that had left the nest, or "adult" for birds more than one year of age. The latter two groups were distinguished by plumage or by skull characteristics (Miller, 1946).

In the field, all blood smears were taken by clipping the center toenail of the bird to obtain a drop of blood. Most nestling and trapped birds were banded and released after being sampled. A number of these were recaptured later, making it possible to study the course and duration of the parasitemia in individual birds. In 1946, serial blood smears were made on banded nestlings at 6 days of age, or 8 or 10 days and occasionally at 12 to 15 days just before they left the nest.

A representative sample of immature birds was selected from trapped specimens and taken to the laboratory for further study. In the laboratory, blood for smears was obtained from most of the birds by puncturing a vein in the leg or wing. In a few cases smears were made with blood from the heart of dead birds. In 1948, samples were taken of peripheral blood of a number of birds prior to sacrificing them, and heart blood and lung smears were made from the same individuals after death.

Examination of blood smears of canaries subinoculated with blood from wild birds is a more sensitive method of detecting *Plasmodium* than is the examination of single peripheral blood smears of the naturally infected wild birds (Manwell and Herman, 1935; 1935a). This xenodiagnosis yields a much truer picture of the prevalence of infection. Therefore, immature birds (occasionally adults as well) of a number of species were so tested. Clarification of parasite identifications and the isolation of parasite strains were not the primary purpose of this study. It is recognized that this procedure does not necessarily present the complete answer. *Plasmodium* can occur in birds without parasitemia (Huff and Coulston, 1946) and thus infection would not be evident in such canaries by the technique employed.

Blood for inoculation into canaries usually was drawn from superficial blood

vessels of either the leg or wing, and, in some cases, from the heart. Of 269 canaries inoculated, all but 29 were inoculated by the intramuscular route, 24 by intravenous route and 5 by a combination of the two routes. The amount of inoculum usually was 0.1 ml. of blood, plus a small quantity of heparin as an anticoagulant. In a few instances, as little as 0.025 ml. or as much as 0.25 ml. of blood was inoculated.

Following injection, smears were made of the blood of the canaries at two- or three-day intervals, at least three smears per week. During 1946, canaries were examined until the smears revealed *Plasmodium* or for a maximum of 60 days. In the other years, smears were made for 60 days whether positive or not.

All canaries were purchased from dealers in San Francisco, California, and were either culls or birds less than a year old. Although natural infections with *Plasmodium* have been reported in captive canaries from southern California (Herman and Vail, 1942), there was no evidence that any of the canaries used in these experiments might have been infected prior to, or during, the course of these experiments except from the donor indicated. All the canaries used in inoculation tests were kept in a screened room, and precaution was taken to prevent accidental infection by mosquitoes.

All smears were stained with Giemsa stain and examined under oil immersion objectives of a compound microscope. All smears were examined for at least 10 minutes before the result was considered negative. Only parasitemias in which the organisms could be identified with certainty are reported. An attempt was made to assign each organism to the proper species. When specific characteristic organisms were absent but schizogonic stages definitely could be recognized, the parasites are considered simply as *Plasmodium* sp. If only trophozoites (which could justifiably be identified as *Haemoproteus* sp. or *Leucocytozoon* sp. as well as *Plasmodium*) or only elongate gametocytes were present (which could justifiably be identified as a *Haemoproteus* as well as a *Plasmodium*), these findings have been omitted from consideration. Additional details of morphological characteristics used for species identifications are presented in a following section where the records of different species of *Plasmodium* are discussed.

The course of a plasmodial infection in birds usually is characterized by an incubation period of from several days to a few weeks, a period of parasitemia which may vary from as short a time as 1 or 2 days to a week or more, and a period of latency which presumably lasts for the life of the host. During latency, relapses may occur with parasitemia lasting for varying periods. During such relapses the blood stream infection is usually of much lower intensity than in the primary instance. Parasitemias observed in birds of the year are interpreted as probable initial infections rather than relapses, but those in adult birds cannot be thus classified because they could represent either one or a combination of new and old infections.

RESULTS AND DISCUSSION

A total of 10,459 blood smears from 8,674 birds, representing 73 species, were examined. Of these, 1,094 smears from 888 birds, representing 27 species, con-

tained *Plasmodium*. In 1946, 3,857 blood smears were made from 3,434 birds of 54 species; in 1947, 3,056 smears from 2,853 birds of 46 species; in 1948, 2,279 smears from 1,281 birds of 27 species; in 1949, 642 smears from 501 birds of 23 species; in 1950, 244 smears from 224 birds of 8 species; and in 1951, 381 smears from 381 birds of 14 species.

Table 1 lists the species found infected with *Plasmodium* during the six-year study. Due to the nature of avian malaria, this is not to be interpreted as its incidence or prevalence in the birds examined. In most cases the figure represents the result of examination of a single blood smear; in some cases of 2, 3 or more smears procured at varying intervals.

The number and species of birds in which no plasmodia were found were: 11 Anthony green herons (*Butorides virescens anthonyi*), 52 Black-crowned night herons (*Nycticorax nycticorax hoactli*), 1 American bittern (*Botaurus lentiginosus*), 1 muscovy duck (*Cairina moschata*), 4 mallards (*Anas platyrhynchos platyrhynchos*), 5 sharp-shinned hawks (*Accipiter striatus velox*), 1 Cooper hawk (*Accipiter cooperii*), 1 western red-tailed hawk (*Buteo jamaicensis calurus*), 2 red-bellied hawks (*Buteo lineatus elegans*), 1 golden eagle (*Aquila chrysaëtos canadensis*), 7 sparrow hawks (*Falco s. sparverius*), 73 California quail (*Lophortyx c. californica*), 273 chickens (*Gallus gallus domesticus*), 15 ring-necked pheasants (*Phasianus colchicus torquatus*), 50 domestic turkeys (*Meleagris gallopavo domesticus*), 8 black-necked stilts (*Himantopus mexicanus*), 24 spotted doves (*Streptopelia c. chinensis*), 211 hybrid doves (*Streptopelia* spp.), 1 yellow-billed cuckoo (*Coccyzus a. americanus*), 8 barn owls (*Tyto alba pratincola*), 2 Pacific nighthawks (*Chordeiles minor hesperis*), 5 black-chinned hummingbirds (*Archilochus alexandri*), 24 western kingbirds (*Tyrannus verticalis*), 9 ash-throated flycatchers (*Myiarchus c. cinerascens*), 25 black phoebes (*Sayornis n. nigricans*), 22 Say phoebes (*Sayornis s. saya*), 7 horned larks (*Eremophila alpestris*), 1 western crow (*Corvus brachyrhynchos hesperis*), 1 plain titmouse (*Parus inornatus*), 1 white-breasted nuthatch (*Sitta carolinensis*), 1 western house wren (*Troglodytes aëdon parkmanii*), 3 Bewick wrens (*Thryomanes bewickii*), 1 California thrasher (*Toxostoma redivivum*), 1 cedar waxwing (*Bombycilla cedrorum*), 2 orange-crowned warblers (*Vermivora celata*), 8 long-tailed chats (*Icteria virens longicauda*), 3 California hooded orioles (*Icterus cucullatus californicus*), 9 western tanagers (*Piranga ludoviciana*), 5 willow goldfinches (*Spinus tristis salicamans*), 2 green-backed goldfinches (*Spinus psaltria hesperophilus*), 1 savannah sparrow (*Passerculus sandwichensis*) and 1 Lincoln sparrow (*Melospiza lincolnii*).

PARASITE RECORDS

Species of *Plasmodium* observed included *elongatum*, *hexamerium*, *nucleophilum*, *polare*, *relictum*, *rouxi*, and *vaughani*. It is of interest that infection with plasmodia as common as *P. cathemerium* or *P. circumflexum* was not diagnosed in this survey.

Plasmodium relictum. This parasite, by far the most frequently observed, was found in at least 702 of the 888 infected birds; the species of hosts and number of individuals infected are given in Table 1.

TABLE 1

Occurrence of Plasmodium *in avian hosts from Kern County, California, 1946–1951*

HOST		NO. OF SMEARS	NO. OF POSITIVE SMEARS	NO. OF BIRDS	NO. OF POSITIVE BIRDS	SPECIES OF PLASMODIUM AND NUMBER OF BIRDS INFECTED							
Common Name	Scientific Name					*P. relictum*	*P. elongatum*	*P. hexamerium*	*P. nucleophilum*	*P. vaughani*	*P. polare*	*P. rouxi*	*P. sp.*
Killdeer	*Charadius v. vociferus*	52	1	50	1*	1*	—	—	—	—	—	—	—
Rock dove	*Columba livia*	43	2	43	2	2	—	—	—	—	—	—	—
Western mourning dove	*Zenaidura macroura marginella*	403	3	383	3*	3*	—	—	—	—	—	—	—
Red-shafted flicker	*Colaptes cafer collaris*	26	2	22	2*	2*	—	—	—	—	—	—	—
Northern cliff swallow	*Petrochelidon pyrrhonota albifrons*	83	2	82	2	—	—	—	—	—	2	—	—
Western mockingbird	*Mimus polyglottos leucopterus*	23	9	23	9*	9*	—	—	—	—	—	—	—
Western robin	*Turdus migratorius propinquus*	60	2	58	2	1	—	—	—	1*	—	—	—
Western bluebird	*Sialia mexicana occidentalis*	2	1	2	1*	—	—	1*	—	—	—	—	—
Mountain bluebird	*Sialia currucoides*	7	1	7	1*	—	—	—	—	—	—	—	1
California loggerhead shrike	*Lanius ludovicianus gambeli*	44	1	43	1*	1*	—	—	—	—	—	—	—
English sparrow	*Passer d. domesticus*	4151	404	2880	242	234	—†	—	—	—	—	—	8
Western meadowlark	*Sturnella neglecta*	7	1	7	1*	—	—	1*	—	—	—	—	—
Yellow-headed blackbird	*Xanthocephalus xanthocephalus*	224	21	215	20*	20*	—	—	—	—	—	—	—
Redwing	*Agelaius phoeniceus*	185	5	173	5	4	—	—	—	—	—	—	1
Tricolor redwing	*Agelaius tricolor*	148	5	148	5*	5*	—	—	—	—	—	—	—
Bullock oriole	*Icterus b. bullockii*	745	61	693	54*	45*	—	2*	—†	—	—	—	7
Brewer blackbird	*Euphagus cyanocephalus*	264	15	238	14	11	—	4*	—	—	—	1*	—
Dwarf cowbird	*Molothrus ater obscurus*	157	8	119	8*	6*	—	2*	—	—	—	—	—
Pacific black-headed grosbeak	*Pheucticus melanocephalus maculatus*	35	1	33	1*	—	—	1*	—	—	—	—	—
Common house finch	*Carpodacus mexicanus frontalis*	1940	516	1741	484	424	18	53*	9*	2*	—	—	14
Lawrence goldfinch	*Spinus lawrencei*	42	1	42	1*	—	—	—	—	—	—	—	1
Spotted towhee	*Pipilo maculatus*	53	7	42	6	4*	—	1*	—	1*	—	—	—
Western lark sparrow	*Chondestes grammacus strigatus*	363	11	335	10*	9*	—	1*	—	—	—	—	—
Oregon junco	*Junco oreganus*	19	3	19	3*	3*	—	—	—	—	—	—	—
White-crowned sparrow	*Zonotrichia leucophrys*	419	7	343	6	4	—	—	—	—	—	2*	—
Golden-crowned sparrow	*Zonotrichia coronata*	34	2	33	2	2	—	—	—	—	—	—	—
Song sparrow	*Melospiza melodia*	15	2	14	2	2	—	—	—	—	—	—	—

* New host records.

† Parasites demonstrated by other techniques.

Certain newly recognized hosts are worthy of comment. Previously, *Plasmodium* had not been found in columbiform birds from the Pacific coast, although *P. relictum* was reported from the common pigeon and the mourning dove east of the Rocky Mountains (Coatney, 1938). The only *Plasmodium* previously identified in the western mockingbird was *P. cathemerium* (Wood and Herman, 1943); both *P. relictum* and *P. elongatum* had been found in the eastern mockingbird in Georgia (Jordan, 1943). The finding of *P. relictum* in a California loggerhead shrike represents the first observation of this parasite not only in this host but also in the family Laniidae in North America. Although this is the first report of *P. relictum* in redwings, *P. cathemerium*, *P. circumflexum* and an unidentified *P.* sp. have been seen in blood of the eastern redwing (*Agelaius p. phoeniceus*) in Massachusetts and Maryland (Herman, 1938). Several species of *Plasmodium* have been identified from eastern cowbirds (*Molothrus a. ater*) (Herman, 1938a; Thompson, 1943), but none previously from the dwarf cowbird. Other new records are presented in Table 1.

The infection with *P. relictum* in the killdeer exhibited a fairly intense parasitemia. Mature schizonts contained 16 to 24 merozoites. In the California loggerhead shrike, mature schizonts with 24 merozoites were frequently observed.

On the basis of morphology alone, study of infected blood smears from the survey and from experimentally infected canaries presented evidence of different strain types within the species *relictum*, particularly in the smears from English sparrows and house finches and from canaries for which these two hosts served as donors. Pigment varied from fine dust-like to large coarse dots. The size of the gametocytes was also noticeably variable, sometimes filling all available space, with the host cell nucleus completely displaced to the periphery or even expelled. The number of merozoites in the different morphological variants observed ranged from 8 to 14 in one group to as many as 32 in the other, with 24 merozoites being the most frequently observed in the latter. There was no particular evidence that infections with these strains were mixed. No attempt is made here to list the numbers infected with these strains since in most cases the infections were of too low an intensity to allow such a classification.

In a number of blood smears, particularly in intense parasitemias, individual erythrocytes frequently contained more than one parasite, sometimes as many as four. In such instances the parasites were usually oriented in the long axis on one side of the host cell nucleus, displacing the nucleus to the side of the cell rather than to the apex as when the cell harbors only a single parasite.

Plasmodium elongatum. In many cases, because the parasites in a blood smear were few, it was difficult to determine to which species of the elongate gametocyte group it belonged. However, on the basis of specific morphological characters, outlined below, an attempt was made to allocate the parasites to species rather than to report them simply as *Plasmodium* sp.

Plasmodium elongatum develops elongate gametocytes in erythrocytes of the peripheral blood. Rounded segmenters with from 8 to 12 merozoites occur chiefly in the bone marrow and only occasionally in the peripheral blood. However, a diagnosis of infection with *P. elongatum* was not made unless extended examination of the slide revealed at least one typical schizont in conjunction with the

elongate gametocytes. Mixed infections with this parasite and other species having elongate gametocytes would have been overlooked since the absence of schizonts characteristic of other species was used as a further criterion for identifying *P. elongatum*. While little difficulty was experienced in separating *P. elongatum* from *Haemoproteus* spp., since the latter usually have a much more regular outline, nevertheless, parasites identified as *Haemoproteus*, or otherwise not showing stages which would permit an unquestioned diagnosis of *Plasmodium* and therefore are omitted from this report, may have been *P. elongatum*.

P. elongatum appeared, with certainty, in blood smears from house finches only (Table 1). Although it was not recognized in any blood smears from English sparrows, when blood was inoculated into canaries (see below), a few infections were uncovered. Examination of over 4,000 blood smears from English sparrows during this six-year study revealed only one infection with elongate gametocytes and the schizogonic stages were not characteristic of *P. elongatum* so the organism was relegated to *Plasmodium* sp.

Plasmodium hexamerium. The presence of typical elongate gametocytes and a maximum number of six merozoites in mature schizonts were the chief criteria for a diagnosis of *P. hexamerium* infection. It is admitted that, particularly in light or mixed infections, the separation of *P. hexamerium*, *P. vaughani*, *P. nucleophilum* and *P. rouxi* in a survey of this type is difficult and not too reliable. At best it can be interpreted only as the opinion of the observer at the time of examination of the specimens at hand. Very few infections with any of these parasites were encountered in the subinoculated canaries, so that further confirmation of these determinations was limited. None of the individuals that harbored these parasites was used as a donor for canary inoculation specifically to clarify the identifications.

Although the western bluebird and spotted towhee are listed (Table 1) as newly recognized hosts, this parasite had already been found in the eastern bluebird (*Sialia s. sialis*) (Huff, 1935, 1939; Wetmore, 1941) and red-eyed towhee (*Pipilo e. erythrophthalmus* (Jordan, 1943).

Plasmodium nucleophilum and *Plasmodium vaughani*. Eleven house finches harbored *Plasmodium* parasites with elongate gametocytes, with schizonts containing 8 merozoites. In 2 of these the gametocytes were typical of *vaughani*, with the larger of two pigment granules often showing the refractile quality considered characteristic of this species. In the other 9 infections the parasites tended to adhere to the host cell nucleus, but not with any constancy. Usually there were more than two pigment granules. In these 9 infections the organism was tentatively identified as *P. nucleophilum*. Neither *nucleophilum* nor *vaughani* had been found in the house finch previously.

P. vaughani was observed in a western robin and a spotted towhee. Both of these represent new host records although it has frequently been found in the eastern robin, the type host (Novy and MacNeal, 1904; Manwell, 1935).

Plasmodium polare. A few *P. polare* were observed in two cliff swallows. This parasite had been reported to infect cliff swallows of the eastern United States (Manwell and Herman, 1935a; Manwell, 1935a).

Plasmodium rouxi. In the present survey an organism in the blood of a Brewer

blackbird was very similar to *P. rouxi*. Although usually the parasite level during the chronic stage of infection with *rouxi* is relatively high, only a few parasites were observed in the smear from the Brewer blackbird. The mature schizonts contained 4 merozoites, with the 2 characteristic pigment granules. The elongate gametocytes were small and few. Comparison with specimens on a slide from a canary infected with *P. rouxi* contributed by Dr. R. D. Manwell further convinced us that this organism was *P. rouxi*. Similar infections, but with fewer parasites, were observed in two white-crowned sparrows, one with only asexual stages. If these infections be truly *P. rouxi*, both hosts would be new in the list and this would represent the first report of this species in other than the type host and type locality. *P. rouxi* had been reported only in sparrows (*Passer domesticus*) and canaries (Sergent *et al.*, 1928) from Algeria. The canary is readily susceptible by blood transfusion, and *P. rouxi* infections have been studied in this host in several laboratories. The original type host belongs to the family Ploceidae and the Brewer blackbird to the family Icteridae, while both the white-crowned sparrow and canary belong to the family Fringillidae. The Ploceidae is strictly an Old World family although the English sparrow and tree sparrow (*Passer montanus*) have been imported into the Western Hemisphere; the Icteridae is strictly a New World family and the Fringillidae is represented by numerous species in both hemispheres.

Plasmodium sp. In a number of infections, developing segmenters could be recognized but numbers of parasites or stages of development were insufficient to justify allocation of the organisms to a particular species. In most cases the gametocytes were elongate. In only one of the English sparrow infections were elongate parasites seen. Extended examination of this slide revealed a single schizont with six merozoites. Smears from a number of other English sparrows revealed parasites classed as *P.* sp., but observations of smears from these same birds when retrapped within a few days indicated that the infecting organism was most probably *P. relictum*, and they are so recorded. Probably plasmodia in the other seven English sparrows reported as *P.* sp. were also *relictum*. As pointed out by Micks (1949), this parasite has been the one most frequently found in all surveys of this host in North America, and in the present survey, except the one infection with elongate gametocytes cited above and a few infections with *P. elongatum* uncovered by inoculation into canaries, it was the only species observed in 242 infected birds.

BIOLOGICAL CONSIDERATIONS

The main features of the life history of avian *Plasmodium* have long been recognized and are familiar to all malariologists. Following injection of sporozoites by mosquitoes or of trophozoites by blood transfusion, a period of prepatency varies from several days to a few weeks, probably according to species of host, species of parasite, stage of parasite and amount of inoculum In most avian infections this period is likely to be under two weeks, although it has lasted as long as two months with *P. polare* (Manwell, 1936). This stage is followed by the patent period when parasitemia occurs and the erythrocytic stages of the

parasites can be observed in blood smear preparations. The duration of this period also varies; parasites may be evident on smears for a single day or two or may persist for a week or more. The infection then becomes latent and presumably lasts for the life of the host. During latency, relapses evidenced by parasitemia may occur and last for varying periods. The subject of relapse in avian malaria was studied by Manwell (1929) who learned that relapses are likely to be short in trophozoite-induced infection of birds, and are usually much less intense than the primary infection. It is generally assumed that the probability of recapturing a bird during a relapse of a magnitude sufficient to exhibit a parasitemia is very small. Manwell (1934), studying 5 species of avian *Plasmodium* by examining single blood smears for several hours, showed that parasites persisted in the body of the host for as long as three years. In the current studies this was not practical, and the several techniques described in the section on methods were selected. The data obtained allow the elucidation in part of several biological aspects of *Plasmodium* infection in a wild bird population.

Multiple smears. A cursory examination of the first 4 columns in Table 1 may seem to indicate that making several smears of blood from a single bird at different times frequently increases the number of birds found to be infected with *Plasmodium*. However, for the most part, smears of blood from the same individual on different days rarely yielded the organisms if they had not been found before.

A large number of birds were sampled either as nestlings or as young of a few weeks of age and were resampled some days or weeks later. Such sampling slightly increased the percentage of known positives but was of greatest value in improving accurate species identification. Of 140 English sparrows captured more than once in 1948 (a few as many as 8 times during a 3-month period), only 13 birds had parasitemia shown in subsequent smears when organisms had not been found in earlier smears. All but one of these were originally sampled as nestlings and were resampled while still in the nest or when trapped within a few weeks.

On the basis of multiple smears, particularly of blood from English sparrows and house finches, most *Plasmodium* infections observed were of short duration. Many birds found to be infected when first examined did not have parasitemia demonstrable in subsequent smears a month or more later. However, there were exceptions. The following were all infected with *P. relictum*: 1 English sparrow examined in July, August and October of 1948 had parasitemia at each sampling; 3 house finches sampled in June or July of 1 year and in the same months again the next year exhibited parasitemia both years; and 1 English sparrow exhibited parasitemia in June for 3 successive years.

Another technique employed, generally considered to disclose more precisely the number of infected birds, was study of smears from heart and lung as well as peripheral blood. In 1948 a sample of birds, including 232 English sparrows, was sacrificed as part of the virus studies. Of the 232 sparrows, 5 were nestlings, 219 immatures and 8 adults. Of the 21 infected with *P. relictum*, 20 were immature birds. In 14, parasites were demonstrated in all 3 smears, in 5 parasitemia

was shown in only heart and lung smears, in 1 in lung smear only, and in 1 in both heart and peripheral smears. Other species were too few to add any significant data relative to *Plasmodium* incidence.

Results of canary inoculations. For the most part, canaries were inoculated with blood only from immature wild birds in which *Plasmodium* had not been found on examination of a direct smear of peripheral blood. This technique uncovered the following infections: *P. relictum* in house finch, English sparrow, song sparrow, spotted towhee, lark sparrow, redwing, Brewer blackbird, and Bullock oriole; *P. elongatum* in house finch and English sparrow; *P. hexamerium* in house finch and Brewer blackbird; and *P. nucleophilum* in Bullock oriole.

A picture of incidence, or number of birds becoming infected within a given season, was obtained. During 1946, 1947 and 1948, 327 immature birds of 7 species were examined and plasmodia were found on single smear examination in 69 of them. Blood from the remaining 258 birds was injected into canaries, provoking subsequent infection in 77. Thus, of the 327 immature birds studied, a total of 146 infections were uncovered by use of both single smear examination and subinoculation into canaries. Results of these studies are presented in Table 2. In addition, blood samples of 11 adult lark sparrows, none of which showed any evidence of *Plasmodium* infection in single smear examination, were subinoculated into canaries with subsequent disclosure of *P. relictum* in 5 of the birds. This is a much higher proportion than was found by other methods (Table 1).

The prepatent period of *P. relictum* in canaries infected by intramuscular inoculation with blood from 80 wild birds varied from 3 days to as long as 26 days, most falling within the range of 4–10 days. The duration of parasitemia varied from 1 day (parasites appearing in only a single smear) to more than 54 days. In the 24 canaries inoculated by the intravenous route the prepatent period for *P. relictum* varied from 4 to 10 days. As only 3 smears a week (taken at 2 or 3 day intervals) were examined, the above periods represent only observed times and might have varied by 1 or 2 days if more frequent examinations had been made.

TABLE 2

Plasmodium *infections in immature birds. (Single smear examination and canary inoculation)*

BIRD	NUMBER OF POSITIVES			
	Number examined	Single smear examination	Additional infections revealed by canary inoculation	Total
House finch	136	46	41	87
English sparrow	169	18	28	46
Bullock oriole	18	2	1	3
Brewer blackbird	22	3	4	7
Redwing	5	0	2	2
Song sparrow	2	0	1	1
Spotted dove	5	0	0	0
Total	327	69	77	146

P. elongatum infected 12 canaries inoculated with blood from wild birds; in all but one of these it occurred with *P. relictum*. The prepatent period in this one canary was 4 days. *P. nucleophilum* and *P. hexamerium* occurred only with *P. relictum*, so it was not possible to determine the beginning of parasitemia in either with any certainty.

Although it may or may not be of significance in relation to possible mortality from *Plasmodium* infection in wild birds, it is interesting that of 111 infections in canaries, mostly *relictum*, infected by inoculation of blood from wild birds or canaries infected from this source, 31 fatalities were attributed to the induced infection. Deaths occurred at the peak of parasitemia. Several other deaths prior to the severe parasitemia may also have resulted from the induced infection. In contrast, as will be reported (Reeves, Herman *et al.*, 1954), when canaries were inoculated with sporozoites from infected mosquitoes, the death rate was even higher: mosquito feeding, 90 per cent; inoculation by syringe, 65 per cent.

Age distribution of infection. Prevalence of infection determined on the basis of direct smear examination in the three age groups is presented in Table 3. Only three species, Bullock oriole, English sparrow and house finch, were numerous enough to warrant such presentation.

Since both relapses and initial infections may be accompanied by parasitemia, *Plasmodium* infections in nestlings and young of the year are likely to be a truer picture of incidence of new infections than are all infections including those in adult birds.

There are few records of *Plasmodium* infections revealed by blood smear examination from birds as young as nestlings. Herman (1938) reported that in Massachusetts blood stream infection of redwings did not occur until after they left the nest. Subsequently, he observed that yellow-billed magpies (*Pica nutalli*) and crows (*Corvus brachyrhynchos hesperis*) in northeastern California usually became infected while still in their nests (Herman, 1952). In the present study, primarily in 1946 and 1947, blood smears from nestlings of a number of species of birds were examined and found to be infected. Some of the following observations indicated that many of the birds were infected shortly after hatching.

In the English sparrow, 799 nestlings were sampled from over 300 nests during the first four years of this study. Seven were found infected with *P. relictum*. The age of each bird tested was recorded and it is felt that these data are accurate to within one day. The birds left their nests from the 11th to the 15th day.

TABLE 3

Prevalence of Plasmodium *by age groups (Single blood smear examinations, 1946–1951)*

BIRD	ADULT			IMMATURE			NESTLING		
	Total	Positive		Total	Positive		Total	Positive	
		No.	Per cent		No.	Per cent		No.	Per cent
Bullock oriole....	303	28	9.2	321	26	8.1	39	0	0.0
English sparrow..	297	28	9.4	1864	198	10.6	799	7	0.9
House finch.....	661	226	34.2	517	198	38.3	494	38	7.7

Smears were made from the blood of birds from 4 to 16 days after hatching. The ages of the 7 infected nestlings ranged from 7 to 13 days. Although two nests with 3 young, and two nests with 4 young contained infected birds, *Plasmodium* was not observed in more than one of the siblings from any English sparrow nest.

Smears were made from the blood of 494 house finches from more than 200 nests; 33 were infected with *P. relictum*. The nestlings examined varied in age from 4 to 16 days and infection was found in some birds only 8 days old. On several occasions smears of more than one of the siblings were positive. In the 51 nests with only a single young, only 2 birds were infected. Of 50 nests with 2 birds, young were infected in two of the nests; parasitemia was present in one sibling in one nest and in both siblings in the other. Of 59 nests examined with 3 young in each, 10 nests had positive birds; 3 nests with only one bird infected, 3 nests with 2, and 4 nests in which all 3 occupants had parasitemia. Twenty-nine nests had 4 siblings each, with infected birds in 6: there were 4 with only one of the young and 2 with 2 siblings from each infected. Organisms were not found in any of the birds from 6 nests with 5 siblings.

The only other fringillid nestling in which parasitemia was observed was the Lawrence goldfinch. During 1946 and 1947, 41 birds 7 to 14 days old from 16 nests were examined and one individual in a nest of 4 at 12 days of age was found infected with *Plasmodium* sp.

Early infections were observed in several species of icterid birds. In 1946, *P. relictum* was identified in the birds from 14 of 109 yellowheaded blackbird nests examined and in 16 of the 199 nestlings. The youngest bird having parasitemia was a 7-day-old, and in 2 cases both siblings of nests containing 2 young were found infected with *Plasmodium* on the 9th day after hatching. Brewer blackbirds were found infected as early as the 7th day, and a redwing harbored *P. relictum* on the 10th day. One nestling redwing in which organisms were not found on the 8th day after hatching was infected with *P. relictum* when trapped and resampled at 18 days of age. *P. relictum* was observed also in nestling tricolor blackbirds.

A robin had *P. relictum* in its blood on the 10th day after hatching, a killdeer on the 6th day and a California shrike on the 13th day. The incidence of *P. relictum* in nestlings was highest in the mockingbird. Of 12 nestlings from 5 nests, 8 had parasitemia. The youngest bird found infected was an 8-day-old. Two nests contained 3 siblings each, all infected.

P. polare was observed in smears from 2 nestling cliff swallows. Manwell and Herman (1935) found this parasite in nestling cliff swallows in New York.

A few nestlings of other species, including 39 Bullock orioles, were examined with negative findings during this study.

Yearly and seasonal incidence. The percentage of blood smears positive for *Plasmodium* in all birds sampled varied considerably from year to year. However, since different species of hosts predominated in our samples each year, a more accurate indication of possible seasonal variations is afforded only when analysis is confined to the few species adequately represented throughout the study.

Since infection in adults may represent a relapse, only data on the incidence

TABLE 4

Yearly variation of Plasmodium *parasitemia prevalence in immature birds. (Single blood smear examinations)*

YEAR	BIRD	SMEARS		
		Total	Positive	
			No.	Per cent
1946	Bullock oriole	161	5	3.1
	English sparrow	388	42	10.8
	House finch	302	118	39.0
1947	Bullock oriole	81	4	4.9
	English sparrow	502	53	10.6
	House finch	105	25	23.8
1948	Bullock oriole	11	1	9.1
	English sparrow	924	115	12.4
	House finch	16	4	25.0
1949	Bullock oriole	44	5	11.4
	English sparrow	145	20	13.8
	House finch	18	11	61.1
1950	Bullock oriole	9	1	11.1
	English sparrow	51	14	27.4
	House finch	68	35	51.5
1951	Bullock oriole	14	10	71.4
	English sparrow	5	2	40.0
	House finch	30	7	23.3

in young of the year is presented here. Each peripheral smear from trapped birds was considered as one sample; thus, if 2 or 3 smears were procured from a single individual on different days within a given month, each smear, not each bird, was tabulated. Except for a number of birds sacrificed in 1948 when lung and heart smears were obtained, all examinations were of peripheral blood smears. To keep all data comparable, peripheral smears of these birds were used to estimate seasonal incidence.

There is some evidence (Table 4) that an increase or decrease in infection in one species is paralleled by an increase or decrease in the other two species.

Most parasitemias were observed in June, July, and August. In the total series for the six-year period the greatest incidence in English sparrows occurred in July and August. The incidence in all summer months was higher in house finches than in English sparrows (Table 5). Few birds were examined at earlier or later dates.

In English sparrows in 1946 and 1947 the incidence of parasitemia was significantly higher in July than in August, while in 1948 the reverse was true. The peak of production of young of English sparrows was in June of 1946, 1948 and 1949, and in May of 1947.

Inoculation into canaries of blood from immature birds in which parasitemia had not been found, as pointed out earlier, reveals more infections than examination of direct peripheral smears from the wild host. Infections of canaries (Table 6) by inoculation of blood from immature English sparrows and house finches

TABLE 5

Monthly prevalence of parasitemia in immature English sparrows and house finches. (Single blood smear examinations)

YEAR		ENGLISH SPARROW					HOUSE FINCH*			
		May	June	July	Aug.	Sept.	May	June	July	Aug.
1946	Number smears	102	43	127	74	40	5	85	155	50
	Number positive	1	2	31	8	0	2	41	54	21
	Per cent positive	1.0	4.6	24.4	10.8	0.0	40.0	48.2	34.8	42.0
1947	Number smears	98	111	147	128	12	3	25	42	35
	Number positive	2	12	32	7	0	0	4	6	15
	Per cent positive	2.0	10.8	21.8	5.5	0.0	—	16.0	14.3	42.8
1948	Number smears	64	234	322	174	123	—	2	13	1
	Number positive	0	4	31	65	14	—	0	4	0
	Per cent positive	0.0	1.7	9.6	37.4	11.4	—	—	30.8	—
1949	Number smears	24	78	33	10	—	—	2	16	—
	Number positive	2	8	7	3	—	—	1	10	—
	Per cent positive	8.3	10.2	21.2	30.0	—	—	—	62.5	—
1950 and 1951	Number smears	—	11	3	27	15	1	29	39	30
	Number positive	—	0	2	7	7	0	7	15	20
	Per cent positive	—	0.0	—	25.9	46.7	—	24.1	38.5	66.7
Total	Number smears	288	477	632	413	190	9	143	265	166
	Number positive	5	26	103	90	21	2	53	89	56
	Per cent positive	1.7	5.4	16.3	21.8	11.0	22.2	37.1	33.6	48.3

* In September the number of house finches sampled was insufficient to warrant analysis.

TABLE 6

Prevalence of Plasmodium *at different time periods in immature birds. (Infection rates determined by canary inoculation of blood)*

DATE COLLECTED		ENGLISH SPARROWS*			HOUSE FINCH		
		Total	Positive		Total	Positive	
			No.	Per cent		No.	Per ceet
June 20–30	1947	20	8	40.0	10	8	80.0
July 1–14	1946	14	9	64.3	4	4	100.0
	1947	1	1	—	5	4	80.0
	1948	23	0	0.0	6	3	50.0
July 15–28	1946	19	11	57.9	42	40	95.2
	1948	11	1	9.1	6	1	16.7
July 29–	1946	8	6	75.0	—	—	—
Aug. 11	1947	6	1	16.7	23	19	82.6
	1948	13	1	7.7	1	0	—
Aug. 12–26	1947	22	2	9.1	7	5	71.4
	1948	18	5	27.8	3	3	100.0
Total		155	45	29.0	107	87	81.3

* In the period August 27 to September 10, 1948, 9 specimens examined and negative, and from September 11 to 24, 1948, 6 specimens examined and 2 positive.

with no demonstrable parasitemia at the time of bleeding further accentuate the evidence of high infection rates based on direct smear examination. The high prevalence of *Plasmodium* infection in the house finches, even as early as the last week in June, leads one to assume that very few individuals of this species escape infection.

The data on English sparrow and house finch nestlings may partially explain the difference in infection rates between these two species. Although blood samples from English sparrows were adequately numerous from April to August, parasitemias were observed only during May and June when the greatest numbers were examined. House finch nestlings, however, had parasitemia throughout the summer: of 46 blood smears examined in April, 1 was positive; of 201 in May, 6 were infected; of 125 in June, 8 were positive; of 104 in July, 16 were positive; and of 12 in August, 2 were positive.

Habitat relationships. The bird hosts examined were obtained from four major habitat areas. These habitats represented distinct biota, from the standpoint both of species and numbers of birds and of differences in mosquito vector populations. Details of the ornithological aspects of the problem will be discussed in a separate publication (McClure, 1952). However, certain general and specific factors possibly affecting the findings must be considered.

Table 7 is a tabulation of the findings in all blood smears examined from wild birds from the 4 major areas during this study. Because of variations in different areas in the numbers, age and species of birds examined, and the inclusion of species in which parasites were not observed, these figures can be interpreted only as trends, not as an accurate picture of incidence or prevalence.

During this study, 33 species of birds were sampled from Hart Park and 18 were found infected with *Plasmodium*; 11 species from the Olive Grove, of which 5 were infected; 18 species from Shafter, of which 7 were infected; and 13 species from within the city limits of Bakersfield, of which 3 were infected. English sparrows were taken primarily from Shafter, and a large sample was also taken from the urban area of Bakersfield. They were extremely rare in Hart Park and

TABLE 7

Trend of annual parasitemia prevalence in wild birds of all ages and species from four areas. (Single blood smear examinations)

YEAR	OLIVE GROVE			HART PARK			SHAFTER			URBAN		
	Total	Positive		Total	Positive		Total	Positive		Total	Positive	
		No.	Per cent		No.	Per cent		No.	Per cent		No.	Per cent
1946	682	122	17.9	1751	187	10.7	797	37	4.6	198	2	1.0
1947	536	35	6.5	1386	117	8.4	725	64	8.8	247	8	3.2
1948	19	6	31.6	252	37	14.7	994	92	9.2	522	15	2.9
1949	0	0	—	235	49	21.0	402	22	5.5	4	0	—
1950	40	12	30.0	71	31	43.7	30	14	46.7	97	7	7.2
1951	178	28	15.7	—	—	—	59	20	33.9	140	0	—
Total	1455	203	14.0	3695	421	11.4	3007	249	8.3	1208	32	2.6

TABLE 8

Trend of annual parasitemia prevalence in four areas. (Combined results of single blood smear examination of immature house finches and English sparrows)

YEAR	OLIVE GROVE SMEARS			HART PARK SMEARS			SHAFTER SMEARS			URBAN SMEARS		
	Total	Positive		Total	Positive		Total	Positive		Total	Positive	
		No.	Per cent		No.	Per cent		No.	Per cent		No.	Per cent
1946	164	64	39.0	138	54	39.1	65	10	15.4	149	0	0.0
1947	46	8	17.4	57	16	28.1	307	51	16.6	134	1	0.7
1948	2	0	—	11	4	36.4	536	98	18.3	386	17	4.4
1949	—	—	—	18	11	61.1	145	20	13.8	—	—	—
1950	28	12	42.8	36	19	52.8	21	10	47.6	30	4	1.3
1951	29	7	24.1	—	—	—	3	2	66.7	—	—	—
Total	269	91	33.8	260	104	40.0	1077	191	17.7	699	22	3.1

the Olive Grove. In 1946, and to a lesser extent in 1947, many English sparrows were sampled from an area almost directly across the Kern River from Hart Park and these were included in the Hart Park tabulations although, strictly speaking, the English sparrow was not an important constituent in the biota of this area. Of 162 English sparrows sampled in 1946 from this area, 32 (19.8 per cent) were infected, and infected birds were not observed among the 28 English sparrows sampled in 1947. No English sparrows were trapped in this area during the subsequent years. The house finch, on the other hand, was primarily a species of the Park and Olive Grove and was of no significance in the other two major areas. The findings in the immature birds of these 2 significant species are presented in Table 8.

Only 4 species of birds were sampled from *all* 4 areas: English sparrows, house finches, Brewer blackbirds and California loggerhead shrikes. Parasitemia was observed in English sparrows from all 4 areas. Parasites were observed in house finches in all but the urban area, where very few of this species were sampled. This species included a high percentage of individuals with parasitemia and accounted for a good number of the infections observed at both Hart Park and Olive Grove. Similarly, the English sparrows accounted for most of the infections observed in our samples from Shafter.

As a further example of the variations in the occurrence of bird species in different areas, 3 species of birds were sampled from the Park, Olive Grove and Shafter: Bullock oriole, mourning dove and kingbird. One species—western lark sparrow—was taken in the Park, Olive Grove and urban areas. Other birds were taken in only one or two of the study areas or from other areas not within the four major locations. Numbers of these birds sampled and the infection rates do not warrant individual analysis.

A closer approximation to the actual prevalence of *Plasmodium* in the two most abundant species of birds—English sparrows and house finches—found in various habitats is obtained by reconsideration of a part of the canary inoculation data. The infection rates expressed in Table 9 are based on single smear

TABLE 9

Plasmodium *prevalence rates in house finches and English sparrows by area. (Combined results of single blood smear examinations and canary inoculations)*

SPECIES AND AREA	1946			1947			1948			TOTAL		
	Total	Positive		Total	Positive		Total	Positive		Total	Positive	
		No.	Per cent		No.	Per cent		No.	Per cent		No.	Per cent
House finches												
Olive Grove	24	24	100.0	16	15	93.8	2	0	—	42	39	92.8
Hart Park	22	20	90.9	29	21	72.4	11	7	63.6	62	48	77.4
English sparrows												
Olive Grove	3	2	66.7	4	0	—	1	0	—	8	2	25.0
Hart Park	22	15	68.2	6	1	16.7	—	—	—	28	16	57.1
Shafter	16	9	56.2	37	11	29.7	52	8	15.4	105	28	26.7
Urban	—	—	—	—	—	—	26	0	0.0	26	0	0.0

examination plus canary inoculation. A series of immature birds was examined for parasites, and blood from all that were negative on single smear examination was inoculated into canaries. The tabulations represent the aggregate of these findings and, thus, as accurate a figure as was practicable to obtain.

SUMMARY

An epizoological study of *Plasmodium* infections in wild birds of Kern County, California, in the years 1946 through 1951 greatly extended knowledge of the occurrence of these parasites and their behavior in nature. Examination of 10,459 blood smears from 8,674 birds representing 73 species resulted in the observation of *Plasmodium* spp. in 1,094 smears representing 888 individual birds of 27 species. Seven species of *Plasmodium* were found: *relictum*, *elongatum*, *hexamerium*, *nucleophilum*, *polare*, *rouxi* and *vaughani*.

Plasmodium relictum was by far the most frequently observed species, occurring in at least 79 per cent of the infected birds. Twelve new host species are recorded for this parasite. Sufficient morphological variation was observed to indicate that two strains of this species probably exist in nature.

Numerous new host records were made of plasmodia with elongate gametocytes. The finding of parasites believed to be *P. rouxi* in two new host species represents the first record of the occurrence of this *Plasmodium* outside of Algeria.

Multiple smears were obtained from a number of individual birds over varying time periods. Evidence of prolonged parasitemia was unusual, but some individuals had parasitemia on consecutive months and even for three successive years. In most individuals, parasitemias were of short duration.

The inoculation of blood from wild birds into canaries led to the demonstration of many infections not observed on blood smear examination of donors. Use of these two complementary techniques led to more complete host records and a truer picture of the prevalence of infection.

Three age classes of birds were studied—nestling, immature (less than 1 year of age) and adult. Parasites were observed in all three groups but infections in

the younger individuals were most susceptible to interpretation. As to time of onset, numerous records were obtained of infection in nestling birds. Prevalence rates in immature birds after a single season's exposure ranged from 64 to 100 per cent in the house finch and 17 to 68 per cent in the English sparrow in different areas and years.

Marked differences were found in the prevalence rates in different summer months, years and areas. It is believed these differences reflect variation in a number of environmental factors.

This study indicates the extensive distribution of *Plasmodium* infection in a wide range of wild avian hosts. The observations are of possible importance in epidemiological studies of other arthropod-borne diseases such as the viral encephalitides for which these birds serve as hosts.

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STUDIES ON AVIAN MALARIA IN VECTORS AND HOSTS OF ENCEPHALITIS IN KERN COUNTY, CALIFORNIA.

II. Infections in Mosquito Vectors[1, 2]

W. C. REEVES, R. C. HEROLD,[3] L. ROSEN,[4] B. BROOKMAN[5] AND W. McD. HAMMON[6]

The preceding paper of this series described a survey during the summers of 1946 through 1951 on the incidence and species of *Plasmodium* in birds of Kern County, California (Herman *et al.*, 1954). The following species of malarial parasites were found in avian hosts of this area: *Plasmodium relictum*, *P. elongatum*, *P. hexamerium*, *P. vaughani*, *P. nucleophilum*, *P. rouxi* and *P. polare*. The present paper reports the occurrence of natural infection with avian plasmodia in mosquito vectors in the years 1946, 1947, 1949 and 1950 in the same area. These studies are a part of extensive observations on the interrelationships of mosquito vectors and avian hosts of the viral encephalitides. This investigation was initiated primarily to clarify the frequency of mosquito vector feedings on the multitude of wild avian species which may serve as hosts of the viral encephalitides as well as of plasmodia infections. The precipitin test for determining the feeding range of mosquito vectors, so useful in other studies, was obviously impractical in the present problem, primarily because of the number of host species and technical limitations of the serological test.

A review of the literature at the onset of the investigations failed to reveal any previous studies under natural conditions on infection rates of mosquito vectors of avian malaria, although the number of papers on field and laboratory studies of this group of parasites is extensive. During and following these investigations reports have been published on two similar smaller surveys in other areas which were stimulated by this project (Grundman *et al.*, 1952; Reeves *et al.*, 1952).

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A total of 21 species of mosquitoes has been recognized (Brookman and Reeves, 1952) in Kern County. In the San Joaquin Valley area of the county it seemed probable that, at most, 7 of these species* were sufficiently common to be considered as potentially important vectors of the malaria infections encountered. Of these 7 species only *Culex tarsalis* and *Culex quinquefasciatus* had been capable of serving as vectors under experimental conditions (Hewitt, 1940). These two species and *Culex stigmatosoma* feed frequently on avian hosts (Hammon *et al.*, 1945; Hammon and Reeves, 1944, 1948; Reeves and Hammon, 1944). *C. tarsalis* was the species of primary interest as it has been the principal vector of encephalitis in this area (Hammon *et al.*, 1945; Hammon and Reeves, 1944, 1948) and was the species of which a knowledge of feeding range would be most valuable.

METHODS

During the summer months, adult female mosquitoes were collected by aspirator from natural resting places (not animal shelters) in four rural study areas known as the Olive Grove, Hart Memorial Park, Shafter Farming Area and Smoot's Ranch. With the exception of the last area, these have been described (Herman *et al.*, 1954). Smoot's Ranch is located on the banks of a natural watercourse, Poso Creek. Except for the stream bed and ranch, all the surrounding area is arid. Conditions on the margin of the stream were favorable for development of *C. tarsalis* and a constant large population of adults of this species occurred all through the study. Specimens were transferred to small cages and held for at least 24 hours before dissection to permit freshly or partially engorged mosquitoes to digest blood meals. Standard malaria survey techniques were used in dissection and examination of stomachs and salivary glands for malarial parasites.

In 1946, in an attempt to determine which species of malarial parasites were present in naturally infected sporozoite-bearing mosquitoes, canaries were inoculated with freshly prepared suspensions of positive glands in saline or Locke's solution by subcutaneous, intraperitoneal, intravenous or intramuscular routes. Blood smears from these birds were prepared and examined for parasites every 48 hours for 30 days following inoculation. Additional gland suspensions in physiological saline solution were frozen at minus 78°F. and were held for several weeks prior to inoculation.

In 1947, all sporozoite suspensions were made in saline and inoculated into canaries by the subcutaneous route. The inoculation dose for canaries was less than that employed in 1946, as half of each gland suspension was inoculated into mice in an attempt to isolate encephalitis viruses.

As a control on the dissection and inoculation techniques in the malaria studies, salivary glands from individual laboratory-infected mosquitoes were dissected in saline, canary serum or chicken serum and sporozoite suspensions in these diluents were inoculated into canaries by the subcutaneous, intramuscular or intravenous route. Examinations for blood parasites were made as above.

**Culex tarsalis, Culex stigmatosoma, Culex quinquefasciatus, Aedes dorsalis, Aedes nigromaculis, Anopheles freeborni,* and *Anopheles franciscanus.*

RESULTS OF MOSQUITO DISSECTIONS

A total of 3,364 mosquitoes representing 8 species were examined. Only 3 species, *C. tarsalis*, *C. stigmatosoma* and *C. quinquefasciatus*, were numerous enough to warrant detailed consideration, and these 3 species were believed to be the most probable vectors of avian malaria.

Summer period infection rates for each of the principal species are given in Table 1. Infection rates differed markedly between different years and species whichever criterion was used to determine these rates, whether that of the positive proportion of all specimens examined, midguts with oocysts, salivary glands with sporozoites, or those mosquitoes in which both midguts and salivary glands were examined and found positive. In 1946 and 1947 when ample numbers of all 3 species were examined, *C. stigmatosoma* was the most frequently infected, *C. tarsalis* slightly less frequently, and *C. quinquefasciatus* significantly less frequently than either.

Yearly and seasonal differences in the proportion of infected *C. tarsalis* were quite marked (Tables 1 and 2). Only this one species was examined throughout the study in numbers sufficient to warrant evaluation. While studies were not made in identical calendar periods in all years, the overlap of periods was sufficient to indicate that in 1946 the incidence of infection was highest and most sustained; in 1947 the rate was quite low except for a two-week period in July and early August; in 1949 it was approximately half-way between the rates in the same time periods in the previous years; and in 1950 the level of infection was very prolonged and constant throughout the entire study period. These data emphasize the yearly variations in intensity of infection when considered on a calendar basis independent of environmental factors to be discussed.

The infection rates of *C. tarsalis* varied considerably between study localities and in different years (Table 3). In 1946 and 1947 the Olive Grove and Hart Memorial Park, in that order, had the highest infection rates with Shafter Farm-

TABLE 1

Species and number of mosquitoes dissected and examined for avian plasmodia, Kern County, California, by year of collection

SPECIES	SPECIMENS				MIDGUTS			SALIVARY GLANDS			BOTH MIDGUTS AND SALIVARY GLANDS		
	Year	Total	Infected		Total	Infected		Total	Infected		Total	Both organs infected	
			No.	Per cent		No.	Per cent		No.	Per cent		No.	Per cent
C. tarsalis	1946	467	76	16.3	420	67	16.0	447	31	6.9	400	22	5.5
	1947	464	31	6.7	430	29	6.7	416	12	2.9	382	10	2.6
	1949	435	45	10.3	422	32	7.6	420	26	6.2	407	13	3.2
	1950	708	47	6.6	706	32	4.5	682	30	4.4	680	14	2.1
C. stigmatosoma	1946	81	18	22.2	78	18	23.0	71	5	7.0	68	5	7.4
	1947	85	9	10.6	77	8	10.4	76	3	3.9	68	2	2.9
C. quinquefasciatus	1946	383	8	2.1	370	8	2.2	361	0	0.0	348	0	0.0
	1947	328	6	1.8	309	5	1.6	301	5	1.6	282	4	1.4

TABLE 2

Culex tarsalis *dissected and examined for avian plasmodia, Kern County, California, by date of collection*

WEEK COLLECTED	1946			1947			1949			1950		
	Total	Infected		Total	Infected		Total	Infected		Total	Infected	
		No.	Per cent		No.	Per cent		No.	Per cent		No.	Per cent
6/16–6/22	—	—	—	25	1	4.0	26	4	15.4	53	4	7.5
6/23–6/29	11	5	45.5	97	4	4.1	53	4	7.5	56	5	8.9
6/30–7/6	51	12	23.5	15	1	6.7	0	—	—	89	4	4.5
7/7–7/13	47	9	19.1	29	1	3.4	200	20	10.0	87	6	6.9
7/14–7/20	5	0	0.0	34	1	3.0	156	17	10.9	93	4	4.3
7/21–7/27	54	9	16.7	59	9	15.3	—	—	—	60	5	8.3
7/28–8/3	71	11	15.5	96	12	12.5	—	—	—	94	3	3.2
8/4–8/10	89	19	21.3	45	1	2.2	—	—	—	95	11	11.6
8/11–8/17	93	8	8.6	46	1	2.2	—	—	—	81	5	6.2
8/18–8/24	23	2	8.7	18	0	0.0	—	—	—	—	—	—
8/25–9/1	23	1	4.3	—	—	—	—	—	—	—	—	—
Totals...	467	76	16.3	464	31	6.7	435	45	10.3	708	47	6.8

ing Area and the new area, Smoot's Ranch (1947), following. The trends of differences in infection rates of *C. stigmatosoma* and *C. quinquefasciatus* in the same areas were identical to those of *C. tarsalis*.

The proportion of infected *C. tarsalis* in the four study areas differed in 1949: in Hart Memorial Park and Shafter Farming Area it was the highest and in the Olive Grove it decreased markedly, as it had in Hart Park in 1947. In 1950 all the study areas had comparatively low and quite comparable infection rates, in

TABLE 3

Culex tarsalis *dissected and examined for avian plasmodia, Kern County, California, by year and area of collection*

LOCALITY	1946			1947			1949			1950		
	Total	Infected*		Total	Infected		Total	Infected		Total†	Infected	
		No.	Per cent		No.	Per cent		No.	Per cent		No.	Per cent
Olive Grove	253	57	22.5	112	17	15.2	76	6	7.9	148	10	6.8
Hart Memorial Park	95	13	13.7	101	7	6.9	103	17	16.5	210	18	8.6
Shafter Farming Area	119	6	5.0	68	4	5.9	104	14	13.5	160	7	4.4
Smoot's Ranch	—	—	—	183	3	1.6	152	8	5.3	190	12	6.3
Totals	467	76	16.3	464	31	6.7	435	45	10.3	708	47	6.6

* Parasites observed either in midgut or in salivary glands.

† In 1950, 43 *Culex tarsalis* from other areas of the County were examined, one of which was infected. These are not considered in the text.

contrast to previous years when incidence of infection between areas varied more markedly.

None of the other *Culex* species was examined in 1949. In 1950 one of 14 *C. stigmatosoma* was infected and none of 35 *C. quinquefasciatus*. Over the four-year period the following other species from the same areas were examined: *Anopheles franciscanus*, 205; *Anopheles punctipennis*, 7; *Anopheles freeborni*, 27; *Aedes nigromaculis*, 82; and *Aedes dorsalis*, 43. Of these, 2 *A. franciscanus* contained what appeared to be young pigmented oocysts on the midgut walls. Results of examination of all other stomachs and glands were negative.

IDENTIFICATION OF PARASITES

1946. Sporozoite suspensions from 35 naturally infected mosquitoes collected in 1946 were inoculated into canaries. Of these 35 canaries, 31 lived a sufficient time (30 days) for study. Six birds of 24 inoculated with sporozoites from *C. tarsalis* and 3 of 5 with sporozoites from *C. stigmatosoma* all became infected with *P. relictum*. Twelve frozen suspensions of sporozoites from these two species of mosquitoes were negative for plasmodia on canary inoculation. Two canaries inoculated with sporozoites from *C. quinquefasciatus* failed to develop infection.

1947. Saline suspensions of sporozoites from 20 naturally infected mosquitoes collected in 1947 were inoculated into canaries; infection was not found in these birds subsequently.

On the basis of the 1946 experience one might have anticipated at least 5 isolations in canaries. However, it is possible that the change in technique in 1947, materially reducing the volume of sporozoite suspension inoculated, affected the results. Immune responses within the canary also may have played a part.

Control studies. The results of studies on different diluents and routes of inoculation using laboratory-infected mosquitoes as the source of *P. relictum* sporozoite suspensions are presented in Table 4.

TABLE 4

Results of the inoculation of sporozoite suspensions from mosquitoes infected from canaries parasitized with Plasmodium relictum

SOURCE OF SPOROZOITES	SUSPENDING MEDIUM AND ROUTES OF INOCULATION									
	Saline			Canary serum				Chicken serum		
	Intramuscular	Subcutaneous	Intravenous	Intramuscular subcutaneous	Intramuscular	Subcutaneous	Intravenous	Intramuscular	Subcutaneous	Intravenous
Culex tarsalis	1/3*	0/1	—	—	2/2	2/2	1/1	2/2	—	1/1
Culex quinquefasciatus	0/1	0/1	—	1/2	—	—	—	—	—	—
Culex stigmatosoma	—	—	2/2	—	—	—	—	—	—	—
Totals	1/4	0/2	2/2	1/2	2/2	2/2	1/1	2/2	—	1/1

* Numerator represents number of positive results, denominator the number of attempts made.

On the basis of this small study it appears that saline may possibly be inferior to other media for transfer of sporozoites from the vector to the avian hosts. Other workers have expressed the same opinion, and further studies are indicated.

DISCUSSION

The demonstration that *Culex* mosquitoes act as important natural vectors of bird malaria confirms the suspicions of many workers in this field. The present study has revealed a marked difference in natural infection rates among the three common *Culex* of Kern County, with evidence that *C. tarsalis* and *C. stigmatosoma* are playing a major role as vectors, and *C. quinquefaciatus* a relatively minor role in that area. The *C. stigmatosoma* population was small during this period and, although it is apparently a most efficient vector, transmission by this one species would not explain the infection rates in avian hosts (Herman *et al.*, 1954). Previously, there were no adequate data to permit evaluation of the vector role of the various mosquito species found in Kern County, California. Actually, *C. quinquefasciatus* has been considered an efficient laboratory vector (Hewitt, 1940). Precipitin test studies on the feeding habits of these three species indicated that they are all aviophilic (Hammon *et al.*, 1945; Hammon and Reeves, 1944, 1948; Reeves and Hammon, 1944). A possible explanation for the observed variation in natural infection rates may be a difference in vector efficiency. Such a difference has been observed in our laboratory and will be reported in the third paper of this series (Rosen and Reeves, 1954).

The marked differences in infection rates in 1946, 1947, 1949 and 1950 are believed to be due in large part to climatic factors. In 1946, the period from June 15 to September 1 was typified by high mean temperatures; between July 1 and August 10 there were only two days in which the daily mean temperatures fell below 78°F., and the maximum mean was 91°F. In 1947 the mean temperatures for the period June 15 to August 31 were comparatively low. Mean temperatures would remain slightly above 78°F. for one to two weeks and then fall below this level. The temperature range in 1949 and 1950 fell between those of the two previous years. As high temperatures are essential for the extrinsic incubation of the parasites in the mosquito vector, this would be expected to affect infection rates. In 1947, there was no apparent decrease in the number of available immature avian hosts or in the mosquito population. The reduced infection rates in the Olive Grove for 1949 and in the Olive Grove, Hart Park and Shafter study areas for 1950 may have been due to a marked decrease in mosquito vector population as a result of intensified mosquito control in those areas. Such a decrease should be reflected in infection rates.

The low infection rate among canaries after inoculation with sporozoite suspensions and the infection of mosquitoes with just one species of parasite (*P. relictum*) may have two explanations. As canaries are known to be susceptible to the other 6 species of malarial parasites demonstrated in this area (Herman *et al.*, 1954), the most logical explanation of the failure to recover species other than *P. relictum* is that the other plasmodia were not sufficiently common to

make it probable that they would be included in the sample of 31 mosquitoes tested in 1946. This is supported by the previously reported studies on incidence of infection of the various species of parasites in avian hosts (Herman *et al.*, 1954). A second possible explanation is that the techniques employed were unsatisfactory. Extensive investigations would be required to determine if this is the case. Information available is insufficient to support the conclusion that the naturally infected mosquito vectors were not transmitting other species of *Plasmodium*.

No evidence available at the present time supports the hypothesis that some of the parasites observed in mosquitoes were developmental stages of species of *Haemoproteus* or *Leucocytozoon* with which the wild birds of this area were heavily infected. Similarly, there is no evidence in the study region that vertebrates other than birds were infected with *Plasmodium*.

The presence of what appeared to be immature oocysts of an unidentified species of *Plasmodium* in two wild-caught *A. franciscanus* is difficult to interpret. *Anopheles* mosquitoes have been demonstrated experimentally to be susceptible to infection with *P. relictum* from wild birds (Hunninen *et al.*, 1950). However, *A. franciscanus* feeds principally on mammals and rarely on man or birds. Human malaria was not reported in the study area in 1947. This is further proof of the difficulty of interpreting the occasional findings of what appear to be malarial parasites in *Anopheles* species which do not fit the other epidemiological findings in a study of human malaria.

The application of the new knowledge to the feeding habits of *C. tarsalis* and the epidemiology of the viral encephalitides, as well as detailed discussion of differences in intensity of *Plasmodium* infection in various areas and various vectors and the factors contributing to these differences, will be considered in the fourth paper of this series (Reeves *et al.*, unpubl.).

SUMMARY

In a study to determine the natural vectors of avian *Plasmodium* infection in Kern County, California 3,364 mosquitoes were examined for parasites during the summers of 1946, 1947, 1949 and 1950. The following average infection rates were demonstrated: *Culex tarsalis*, 199 of 2,074 (9.6 per cent); *Culex stigmatosoma*, 28 of 180 (15.6 per cent); and *Culex quinquefasciatus*, 14 of 746 (1.9 per cent). These differences are believed to reflect variation in vector efficiency. Of 364 *Anopheles* and *Aedes* examined, only 2 *Anopheles franciscanus* were infected with what appeared to be young oocysts of unidentified parasites.

The intensity of infection in the principal vector, *Culex tarsalis*, varied considerably in different years and in different areas. These variations are believed to be due to changeable factors such as climate, the effect of mosquito control on the vector population, and ecological differences between areas.

Plasmodium relictum was isolated from naturally infected *Culex tarsalis* and *Culex stigmatosoma*.

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STUDIES ON AVIAN MALARIA IN VECTORS AND HOSTS OF ENCEPHALITIS IN KERN COUNTY, CALIFORNIA

III. The Comparative Vector Ability of Some of the Local Culicine Mosquitoes[1, 2]

L. ROSEN[3] AND W. C. REEVES

In the course of these investigations *Culex tarsalis*, *Culex stigmatosoma*, and *Culex quinquefasciatus* were incriminated as natural vectors of avian malaria in Kern County, California. Although in previous studies on the feeding habits of these species each fed frequently on avian hosts, the natural infection rates of *C. tarsalis* and *C. stigmatosoma* were significantly higher than that of *C. quinquefasciatus* (Reeves *et al.*, 1954). The following laboratory experiments were undertaken to determine if this discrepancy might be a reflection of inherent difference in susceptibility to infection with the local plasmodia, as well as to learn whether the parasite could be transmitted from bird to bird by the several species of mosquitoes.

METHODS

All of these studies were carried out in the Kern County Virus Encephalitis Laboratory. Mosquitoes were reared in the laboratory from larvae and pupae. They were held in a room in which the daytime temperature varied between 20°C. and 37°C. and was above 30°C. for most of the day.

The avian hosts in the laboratory were canaries (*Serinus canarius*); these were housed in a screened room at all times.

Strains of plasmodia recently isolated in canaries by blood transfer from naturally infected birds trapped in the Kern County area were utilized in all experiments. *Plasmodium relictum*, the predominant species in the area (Herman *et al.*, 1954), was used in most instances. In a few cases it was necessary to use a canary with a mixed infection of *Plasmodium relictum* and *Plasmodium elongatum*. Canaries with heavy gametocyte parasitemia were selected for mosquito feedings.

OBSERVATIONS AND DISCUSSION

A summary of all experimental feeding of mosquitoes on infected canaries is presented in Table 1. *Culex tarsalis* and *C. stigmatosoma* were readily infected,

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[3] Present address: Laboratory of Tropical Diseases, National Institutes of Health, Bethesda, Maryland.

TABLE 1

Infection rates of mosquitoes fed on canaries infected with Plasmodium relictum*

SPECIES	TOTAL FED	INFECTED	
		Number†	Per cent
Culex tarsalis	88	86	97.7
Culex stigmatosoma	12	12	100.0
Culex quinquefasciatus	72	36	50.0
Aedes dorsalis	13	1‡	7.7
Aedes nigromaculis	3	0	0.0
Aedes vexans	1	0	0.0

* A few mosquitoes were fed on canaries with a mixed infection of *P. relictum* and *P. elongatum*.

† Mosquitoes found infected with either oocysts or sporozoites.

‡ Only one oocyst seen.

whereas *C. quinquefasciatus* became infected in only half the trials. A solitary oocyst was found on the midgut of one *Aedes dorsalis*. Attempts to induce some of the local anophelines to feed on canaries were unsuccessful.

Table 2 presents in detail the information obtained in those experiments in which more than one species of mosquito was fed on the same donor canary at the same time. The results are strictly comparable only within the individual experiments, since the degree of gametocyte parasitemia, the species and strain of *Plasmodium*, and the conditions of mosquito holding and dissection varied in each experiment.

All *C. tarsalis* as well as all *C. stigmatosoma* became infected in these experiments. In some cases no *C. quinquefasciatus* became infected, while in others varying infection rates of this species were observed. *Culex quinquefasciatus* became infected only in those experiments in which *C. tarsalis* and *C. stigmatosoma* became heavily infected, as indicated by their average oocyst counts. In all instances the average oocyst counts of *C. quinquefasciatus* were well below those of *C. tarsalis* and *C. stigmatosoma*. It is of interest that sporozoites were found in the salivary glands of one out of three specimens of *C. tarsalis* in as short a time as four days after feeding on an infected canary, and in nine out of ten specimens five days after feeding.

These observations seem to offer a logical explanation for the difference in natural infection rates observed among these three mosquito species (Reeves *et al.*, 1954). Since infected wild birds of the area usually were found to have far fewer gametocytes in their peripheral blood than the experimentally infected canaries chosen for their high gametocyte parasitemia, it is reasonable to anticipate that the difference in infection rates in nature of *C. tarsalis* and *C. stigmatosoma* and that of *C. quinquefasciatus* would be even greater than indicated by the average in Table 1. No appreciable difference in infection rates was noted between *C. tarsalis* and the few *C. stigmatosoma* used in these experiments. Definite conclusions cannot be drawn in regard to *Aedes dorsalis* and the other *Aedes* species because of the small numbers used.

TABLE 2

Infection rates of several species of mosquitoes fed simultaneously on the same canaries infected with Plasmodium relictum

EXPER. NO.	SPECIES	TOTAL EXAM.*	NO. POSITIVE†	MIDGUTS EXAMINED		
				Total‡	Positive	
					No.	Av. oocyst count (±10%)
8S	*C. tarsalis*	4	4	1	1	9
	C. quinquefasciatus	4	0	1	0	—
9S§	*C. tarsalis*	10	10	10	9	17
	C. quinquefasciatus	3	1	3	1	1
	A. dorsalis	1	0	1	0	—
10S	*C. tarsalis*	4	4	4	4	30
	C. quinquefasciatus	5	0	5	0	—
11S§	*C. tarsalis*	17	17	15	15	220
	C. quinquefasciatus	21	17	17	15	29
14S	*C. tarsalis*	3	3	3	3	300
	C. quinquefasciatus	4	4	4	4	90
15S	*C. tarsalis*	5	5	5	5	166
	C. stigmatosoma	4	4	4	4	200
	C. quinquefasciatus	16	3	16	3	15
	A. dorsalis	1	0	1	0	—
16S	*C. tarsalis*	16	16	16	16	171
	C. stigmatosoma	4	4	1	1	25
	C. quinquefasciatus	4	1	4	1	3
	A. dorsalis	11	1	11	1	1
17S	*C. tarsalis*	5	5	5	5	13
	C. stigmatosoma	4	4	4	4	6
	C. quinquefasciatus	2	0	2	0	—

* Mosquitoes were dissected from 4 to 15 days after feeding on infected canary.

† Number showing either oocysts or sporozoites.

‡ Because some mosquitoes in these groups were used for transmission experiments and were dissected while engorged, it was not possible to examine their guts.

§ These canaries had a mixed infection of *P. relictum* and *P. elongatum*.

Table 3 presents the results of bird to bird transmission experiments employing the three incriminated species of *Culex* mosquitoes. All three species were able to transmit *P. relictum* from canary to canary. In the three experiments in which the donor canary was infected with gametocytes of both *P. relictum* and *P. elongatum*, only *P. relictum* appeared to be transmitted to the recipient canary. This apparent failure to transmit *P. elongatum* might be explained by the fact that all three recipient canaries died within eleven days

TABLE 3

Experimental mosquito transmission of Plasmodium relictum

SPECIES OF MOSQUITO	EXPER. NO	NO. OF INFECTED MOSQUITOES FEEDING*	INCUBATION PERIOD IN MOSQUITO, DAYS	PREPATENT PERIOD IN RECIPIENT CANARY,† DAYS
C. tarsalis	1T	1	11	5
	2T	1	12	6
	3T	2	14	6
	4T	1	14	6
	5T	1	15	5
	6T	2	15	5
	7T	14	11	5
	8T	2	20	4
	9T‡	1	12	4
	10T‡	1	12	4
C. quinquefasciatus	11T‡	2	12	4
	12T	1	8	neg.
	13T	1	8	6
C. stigmatosoma	14T	1	6	11
	15T	1	6	6
	16T	1	6	neg.

* All mosquitoes were shown to have sporozoites in their salivary glands by dissection after the feeding on recipient canary.

† Since blood smears on these canaries were examined every other day, the actual prepatent period in some instances is probably shorter than indicated.

‡ These donor canaries had a mixed infection of *P. relictum* and *P. elongatum*. Recipient canary showed only *P. relictum* (see text).

of being bitten by the transmitting mosquitoes. They may have died before the prepatent period of *P. elongatum* had elapsed, since it is known that local strains of this species have a longer prepatent period in the canary than does *P. relictum* (Herman, 1954). It is seen from Table 3 that *P. relictum* had a prepatent period as short as four days in four of the transmission experiments. The ability of *C. quinquefasciatus* to transmit *P. relictum* is well-known (Hewitt, 1940) *Culex tarsalis* can also transmit this species of avian malaria (Huff, 1932). However, this is believed to be the first record of the ability of *C. stigmatosoma* to transmit any species of avian *Plasmodium*.

SUMMARY

Culex tarsalis, Culex stigmatosoma and *Culex quinquefasciatus* were susceptible to infection with and capable of transmitting to canaries local strains of *Plasmodium relictum*.

Culex tarsalis and *Culex stigmatosoma* were more easily infected with these strains than was *Culex quinquefasciatus*. This observation affords an explanation for the difference in natural infection rates observed in other studies.

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COMPARATIVE STUDY OF CHLOROQUINE AND AMODIAQUIN IN THE TREATMENT OF GIARDIASIS

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In Mexico, the incidence of intestinal parasitosis due to *Giardia lamblia* varies from 5 to 33 per cent, but its pathogenicity has until recently been a subject of controversy. *Giardia* is sometimes considered to be pathogenic and consequently there is a search for a specific therapy. Maris and Bushong studied a group of 85 children with giardiasis and found that the most common symptoms in the order of frequency were abdominal pain, weight loss, anorexia, pruritus, and irritability. Some gastroenterologists think the parasite may cause cholecystitis or cholangitis when it reaches the biliary ducts. However, even in the absence of symptoms attributable to the infection it is considered advisable by many doctors to free the patient of *Giardia*.

No very successful drug was found until in 1933 a series of experiments were started using atabrine in the treatment of giardiasis. Since this antimalarial gave good results, it was thought that the newer antimalarials might be effective also, and Basnuevo and Sotolongo (1946) tried chloroquine, paludrine, and pentaquine. Results with chloroquine were encouraging: 83 per cent of the patients were cured. This drug was well tolerated by patients of all ages, and did not have the disadvantage of coloring the skin yellow. Soberon and Pelaez (1948) confirmed the successful use of chloroquine by Schneider and Uzan (1947) in the treatment of giardiasis.

In 1946, the antimalarial amodiaquin was developed. This drug has the advantage of being effective in a single dose, which is of great importance in the treatment of children. Studies in laboratory animals have shown the safety of amodiaquin and clinical reports have confirmed its relative lack of toxicity.

MATERIAL AND METHODS

This study was done in the Laboratory of Parasitology of the I. P. N. (National Polytechnic Institute) and in private institutions. In two groups of 50 each, one hundred patients were studied, 73 males and 27 females ranging in age from 2 to 39 years, and all infected with *Giardia lamblia*, sometimes alone and sometimes associated with other parasites. Each group of 50 patients was treated orally, one with chloroquine, the other with amodiaquin.

Adequate observation of the patients was possible thanks to the interest of the patients themselves, and to the medical services of the I. P. N. Methods of laboratory diagnosis were by direct stool examination and by flotation with zinc sulfate. Stool examinations for *Giardia lamblia* were made one day before and three days after treatment was started, and repeated every four to eight days for a period of one to six months.

Chloroquine was furnished by the Colliere Laboratories. Dosage for adults

TABLE 1

50 patients treated with chloroquine (0.10 gm. tablets)

NO. OF PATIENTS	AGE	SEX	GIARDIA PRESENT	GRAMS × DAYS	TOTAL DOSE	RESULTS OF FOLLOW-UP
11	2–13	2—F 9—M	+ to +++	0.2 × 5	1.0	Negative on 3rd post-treatment day.
25	5–31	8—F 17—M	+ to ++++	0.3 × 5	1.5	Negative on 3rd post-treatment day.
4	11–22	2—F 2—M	+ to ++++	0.3 × 5	1.5	Became negative on 10th post-treatment day.

40 or 80% of 50 patients cleared after 5 day treatment with chloroquine.

Patients who continued positive, re-treatment with chloroquine

NO. OF PATIENTS	AGE	SEX	GIARDIA PRESENT	GRAMS × DAYS	TOTAL DOSE	FOLLOW-UP STOOL EXAM.	RE-TREATMENT GRAMS × DAYS	FOLLOW-UP STOOL EXAM.
4	12–23	2—F 2—M	++ to +++	0.3 × 5	1.5	Positive	0.3 × 5	Negative
3	18–22	1—F 2—M	++ to +++	0.3 × 5	1.5	Positive	0.3 × 5	Positive

Patients who continued positive, not re-treated

AGE	SEX	GIARDIA PRESENT	GRAMS × DAYS	TOTAL DOSE	FOLLOW-UP STOOL EXAM.	
2	F	+++	0.2 × 5	1.0	Positive	Not retreated
10	F	+++	0.3 × 5	1.5	Positive*	Not retreated
3	M	++++	0.2 × 5	1.0	Positive	Not retreated

* Became positive on 15th post-treatment day probably reinfected by family.

TABLE 2

50 patients treated with amodiaquin (0.2 gm. tablets)

NO. OF PATIENTS	AGE	SEX	GIARDIA PRESENT	GRAMS × DAYS	TOTAL DOSE	RESULTS OF FOLLOW-UP
7	2–7	7—M	++ to ++++	0.2 × 2	0.4	Negative.
9	8–12	3—F 7—M	++ to ++++	0.4 × 1	0.4	Negative.
33	13–30	6—F 27—M	+ to ++++	0.6 × 1	0.6	Negative.
1	10	F	+++	0.4 × 1	0.4	Negative. Became positive on 20th post-treatment day.*

* This is one of the relapsing patients in the chloroquine series, and again was judged to be reinfected by family contacts.

was 0.3 gm. daily for five days; for children from 2 to 12 years, 0.2 gm. daily for five days. Total dosage: adults 1.5 gm.; children 1.0 gm. Amodiaquin was furnished by Dr. Soberon and Professor D. Pelaez. Dosage for adults was a single dose of 0.6 gm.; children from 2 to 7 years, 0.2 gm. daily for two days (total 0.4 gm.); children from 8 to 12 years, 0.4 gm. in a single dose; from 13 years on, the adult dose. The data on each case are shown in Tables 1 and 2.

RESULTS AND CONCLUSIONS

Chloroquine was given as indicated, and amodiaquin as a single dose. Neither drug caused any detectable toxic effects. Chloroquine was effective in 44 (88 per cent) of the 50 patients treated with it. It was necessary, however, for 4 of these patients to undergo a second course of treatment in order to eliminate *Giardia* completely. It was considered that 100 per cent success was achieved with amodiaquin. The only doubtful patient was one who had been treated previously with chloroquine and relapsed. This patient also relapsed 20 days after treatment with amodiaquin and was judged in both instances to have been reinfected.

Amodiaquin, administered in single doses,* was more effective than chloroquine in the multiple dosages employed.

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* Seven children from 2 to 7 years were treated with two doses 24 hours apart.

THE USE OF CHLORAMPHENICOL IN RELAPSING FEVER

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Relapsing fever occurring in the area of Pusan, Korea, is louse-borne, its virulence is high, and most patients are extremely toxic and manifest severe prostration, rapid pulse, anemia, and fever ranging from 100°–104°F. A high percentage of the cases have splenomegaly and about 50 per cent show icterus.

The accompanying chart contains data on 27 patients, 5 to 54 years old, whom we were able to observe and examine over varying periods of time after treatment. Fifty-five patients who did not return for blood examination are not reported. It could be assumed that since they did not return they were clinically better All patients were treated with chloramphenicol[1], and in all cases only one day of treatment was required.

In the earlier cases the adult dosage was 2 gm. of chloramphenicol given throughout the day in four doses of 500 mg. each at three hour intervals. Later we found it possible to reduce the dosage to 1 gm. given in four doses of 250 mg. each. The same excellent results were obtained as with the larger dosage. The patient's clinical improvement was rapid in every way and the blood smear was negative on the day following treatment in every patient examined.

Since chloramphenicol can be administered orally, this treatment is of great advantage in a busy clinic.

SUMMARY

Twenty-seven patients varying in age from 5 to 54 years and suffering from virulent relapsing fever were given a one-day treatment with chloramphenicol. All patients were negative for *B. recurrentis* on the second day, and convalescence was rapid. For the treatment of this disease, chloramphenicol is dramatically and rapidly effective even in small doses.

[1] The chloramphenicol (Chloromycetin) used in this study was kindly furnished by Parke, Davis and Company.

Summary of cases of relapsing fever treated with chloramphenicol, Maryknoll Sisters' Clinic, Pusan, Korea

DATE	CASE	SEX	AGE	DURATION OF ILLNESS	TEMPERATURE	ICTERUS	SPLENOMEGALY	BLOOD SMEAR BORRELIA RECURRENTIS		TOTAL CHLORAMPHENICOL
								Pos.	Neg.	
1951										
Aug. 7	A4882	M	15	4 days	104.2	Mild	+	8/7	8/8	2 Gm.
Aug. 8	A4845	M	17	3 days	103.8	+	+	8/8	8/9	2 Gm.
Aug. 13	O.P.P.A.	F	28	6 days	104.	Neg.	+	8/13	8/14	2 Gm.
Aug. 16	O.P.C.L.	F	48	4 days	104.2	Neg.	+	8/16	8/17	2 Gm.
Aug. 17	O.P.K.R.	F	42	5 days	103.8	Neg.	+	8/17	8/18	2 Gm.
Sept. 3	A4845	M	40	3 days	104.	+	+	9/3	9/4	2 Gm.
1952										
Jan. 9	B1828	M	5	4 days	100.2	Neg.	+	1/9	1/10	1 Gm.
Feb. 14	C3761	M	24	3 days	99.8	Neg.	Neg.	2/14	2/15	1 Gm.
Feb. 15	C3905	F	18	5 days	102.	Neg.	+	2/15	2/16	2 Gm.
Feb. 18	4115	M	46	4 days	102.	Neg.	+	2/18	2/20	2 Gm.
Mar. 13	O.P. 317	M	29	4 days	102.	+	+	3/13	3/14	1 Gm.
Mar. 19	O.P. 340	M	36	4 days	103.	+	+	3/19	3/20	1 Gm.
Mar. 19	O.P. 343	M	25	6 days	104.	+	+	3/19	3/20	1 Gm.
Mar. 19	O.P. 344	M	54	4 days	100.	++	+	3/19	3/20	1 Gm.
Apr. 2	O.P. 425	M	18	6 days	100.6	+	+	4/2	4/3	1.5 Gm.
Apr. 2	O.P. 426	F	26	6 days	102.6	+	+	4/2	4/3	1.5 Gm.
July 3	C16883	F	13	7 days	103.2	+	+	7/3	7/4	2 Gm.
July 4	C16935	M	17	3 days	103.4	Neg.	+	7/4	7/5	2 Gm.
July 22	C17839	F	14	6 days	104.	Neg.	+	7/22	7/23	2 Gm.
Aug. 2	B9188	M	10	20 days	104.	+	+	8/2	8/4	2 Gm.
Aug. 23	C20086	M	45	10 days	102.	Neg.	Neg.	8/23	8/24	2 Gm.
Sept. 2	C20743	F	30	3 days	101.4	Neg.	Neg.	9/2	9/3	2 Gm.
1953										
May 18	D7612	F	10	11 days	104.	+	+	5/18	5/19	2 Gm.
June 22	06030	M	8	4 days	103.	Neg.	Neg.	6/22	6/23	0.5 Gm.
June 22	06039	Record lost						6/22	6/23	
June 29	D11033	M	17	7 days	100.8	Neg.	Neg.	6/30	7/1	1 Gm.
July 17	D12128	F	45	5 days	102.	++	+	7/17	7/20	1 Gm.

TREATMENT OF SCHISTOSOMIASIS MANSONI WITH ANTIMONY-a, a'-DIMERCAPTO-POTASSIUM SUCCINATE (TWSb)

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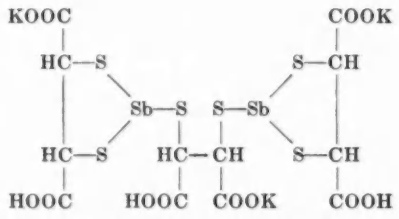
I. CHEMICAL AND PHYSICAL PROPERTIES OF TWSb

TWSb is antimony-a,a'-dimercapto-potassium succinate corresponding to Formula I:



$(C_4H_2O_4S_2K)_2Sb$ MW 898 Sb calc. 27.2
fd. 27.1; 27.3

Trivalent antimony is bound to the sulphur atoms of vicinal dithiols to form pentatomic rings. The compound may be visualized as antimony tartrate in which the oxygen atoms of the hydroxyl groups are replaced by sulphur. As a consequence TWSb differs from tartar emetic as follows: (1) in the pentatomic ring structure of TWSb the antimony is more tightly bound; (2) decomposition by heating leads in tartar emetic to the toxic antimonous oxide, in TWSb to the practically nontoxic antimonous sulphide.

TWSb is a white crystalline powder, soluble in water to more than 30 per cent, insoluble in alcohol, acetone, ether. The analytically pure anhydrous compound is unchanged by heating for one hour at 100°C. The 5 per cent aqueous solution remains unchanged when rapidly boiled up, but becomes discolored and cloudy on prolonged boiling.

II. EXPERIMENTAL DATA

Toxicity and therapeutic effects in laboratory animals

Comparative data concerning the acute toxicity of tartar emetic, fuadin and TWSb in mice are presented in Table 1. The dose cur. 50 s.c. amounts to 0.120 gm./kg. ($\frac{1}{4}\frac{1}{0}$LD50) in *T. congolense* infection (Bisimbi strain) of the mouse (Kershaw, Liverpool*) and the *T. brucei* infection of the guinea pig (Mornet, Dakar*). A dose of 0.5 gm./kg. s.c. and 1.0 gm./kg. *per os* protects against a lethal infection with *T. congolense* (strain Institut Pasteur, Paris) when applied immediately before the inoculum (J. Schneider, Paris).

[1] 333 West 52nd Street, New York 19, N. Y.

* Personal communication.

TABLE 1

1. Acute toxicity of TWSb i.p. in mice compared with tartar emetic and fuadin

COMPOUND	LD 50 I.P.		RELATIVE TOXICITY OF Sb
	Substance gm./kg.	Sb gm./kg.	
Tartar emetic	.05	.018	1/1
Fuadin	.65	.088	1/4.9
TWSb	3.33	.900	1/50

Four daily subcutaneous injections of 0.125 gm./kg. cure an established *Litmosoides carinii* infection of the cotton rat by a lethal effect on the adult worms (Kershaw, Liverpool*). Data supplied by G. Luttermoser, Bethesda*, indicate that in an established *Schistosoma mansoni* infection of the guinea pig 15 per cent of the LD50 of TWSb produces the same reduction of the worm load as 38 per cent of the LD50 of fuadin.

III. CLINICAL DATA

For clinical use 5 to 10 per cent solutions of TWSb were prepared at the time by the addition of sterile distilled water or 5 per cent glucose solution to the anhydrous drug contained in rubber-capped vials. Intramuscular injections were made in volumes not exceeding 5 cc. in the deep gluteal muscles; intravenous injections were given in volumes not exceeding 10 cc. without special precautions as to the speed of the injection, in one to two minutes.

1. Tolerance

A. *Circulatory system*

No case of the 134 patients treated with TWSb presented clinical symptoms of untoward effects on the circulatory system. Observations concerning electrocardiogram and blood pressure follow.

(a) *Electrocardiogram studies*

The effect of TWSb on the ECG was studied in 10 cases, 6 treated by intramuscular and 4 by intravenous injections. Serial ECG's were taken and interpreted by the Cardiac Clinic of the Hospital dos Servidores do Estado, Rio de Janeiro. Following are summaries of the individual reports.

Intramuscular treatment

1. *Male 28 years, 49 kg.* Seven intramuscular injections of TWSb ranging from 0.2 to 0.5 gm., totaling 3.1 gm. (.063 gm./kg.) applied over 12 days. One ECG before, 5 during, 2 twelve and nineteen days after treatment. The serial ECG's show a very moderate transitory modification of the T wave which, in the main leads, is flattened and, on the last day of treatment, negative. Twelve and 19 days after the treatment the amplitude of T has increased again in all

* Personal communication.

main leads and shows a tendency to become positive. A causal relation between the ECG modifications and the medication is possible. In any case, these modifications are insignificant.

2. *Male 38 years, 63.6 kg.* Six daily intramuscular injections ranging from 0.3 to 0.5 gm., totaling 2.7 gm. (.042 gm./kg.); after an interval of 3 days a further dose of 0.5 gm. On the 6th treatment day T is flattened in the main leads, inverted in the right precordial leads. The modifications start to regress 4 days after the first series of treatment, are accentuated 4 days after an additional dose and disappear 12 days after the treatment.

3. *Male 30 years, 62.7 kg.* Seven intramuscular injections of 0.5 gm. TWSb over 10 days (the first four injections daily), totaling 3.5 gm. (.056 gm./kg.). Insignificant decrease of amplitude of T in D2 and VF on 6th day of treatment and 1st and 5th day after treatment. The modifications regress 12 days after the treatment and the ECG is completely normal 19 days after the treatment.

4. *Male 49 years, 56.5 kg.* Seven intramuscular injections of 0.5 gm. TWSb over 11 days, totaling 3.5 gm. (0.062 gm./kg.); the first 5 injections daily. One ECG before (left ventricular hypertrophy), three during and one 3 days after treatment. Transitory insignificant changes in amplitude of T wave and electric position of heart.

5. *Female 30 years, 47.2 kg.* Four intramuscular injections ranging from 0.3 to 0.5 gm., totaling 1.8 gm. (0.036 gm./kg.) given over 4 days. One ECG before (incomplete block of right branch), two during, two 5 and 11 days after treatment. Moderate variation of amplitude of T wave. It is not possible to draw any conclusions as to the possible causal relations between the medication and these variations.

6. *Male 36 years, 50 kg.* Seven intramuscular injections of 0.5 gm., totaling 3.5 gm. (0.07 gm./kg.) given over 11 days, the first 4 injections daily. One ECG before, two during, three 2, 11, and 18 days after treatment. Transitory flattening and inversion of T wave in the right precordial leads (probably in relation with the medication) returned to normal 11 days after the treatment.

Intravenous treatment

7. *Male 40 years, 70.6 kg.* Six intravenous injections ranging from 0.15 to 0.5 gm., totaling 1.85 gm. (0.026 gm./kg.) given daily for 6 consecutive days. One ECG before, one during, two 5 and 11 days after treatment. Transitory decrease of amplitude of T wave mainly in the left precordial leads, returned to normal 11 days after treatment.

8. *Male 32 years, 67.6 kg.* Six intravenous injections ranging from 0.15 to 0.5 gm., totaling 1.85 gm. (0.027 gm./kg.), given daily for 6 consecutive days. One ECG before, three 1, 3, and 13 days after treatment. One day after treatment decrease of T wave and left precordial leads, inversion of T in V1 and diphasic T in V2, V3, and V4. Regression of modification 3 days, and nearly complete return to normal 13 days after treatment.

9. *Male 38 years, 59.6 kg.* Six intravenous injections ranging from 0.1 to 0.5 gm., totaling 2.0 gm. (0.033 gm./kg.), applied daily for 6 consecutive days. One

ECG before, one during, three 3, 8, and 16 days after treatment. Insignificant changes of T wave (moderate flattening) possibly in connection with a change of the electric position of the heart.

10. *Male 24 years, 72 kg.* Single intravenous injection of 1.0 gm. (0.014 gm./kg.). One ECG before, two 1 and 10 days after treatment. Slight decrease of amplitude of T wave immediately after treatment; return to normal 10 days after treatment. This case is significant because it brings out the fact that even a massive dose of TWSb, corresponding in antimony content to 0.7 gm. of tartar emetic, has no significant effect on cardiac function and structure.

It follows that ten cases, treated intramuscularly and intravenously, with various dose schedules of therapeutic level, including one massive single dose, revealed no, or only insignificant and entirely reversible ECG changes, limited to a temporary decrease or inversion of the T wave.

(b) *Blood pressure*

In 35 patients, 7 treated intramuscularly and 27 treated intravenously, the blood pressure was taken before and after every injection for a total of 43 intramuscular injections, ranging from 0.1 to 0.5 gm. and a total of 156 intravenous injections, ranging from 0.2 to 1.0 gm. No significant changes of the blood pressure were found, comparing values before and after individual injections or on consecutive days throughout the treatment.

In this connection the fact may be emphasized that the intravenous injections of TWSb were given without special precautions as to the speed of the injection, within 1–2 minutes. In comparison, intensive treatment with tartar emetic requires intravenous injections to be made in not less than 12 minutes.

B. *Gastro-Intestinal Tract*

A certain discomfort described by patients as "bitter mouth", abdominal discomfort, decrease of appetite, various degrees of nausea, occasional liquid bowel discharges, constipation, were indicated by a number of patients, but these minor and partly subjective occurrences did not lend themselves to precise recording, depending too much on the degree of insistence given to questioning. Frank colics were not observed.

In the following, fifty well observed cases are analyzed, which were submitted to an intensive 2-day treatment with TWSb consisting of 6 intravenous doses given at 8 hour intervals. The total dose ranged from 0.03 to 0.04 gm./kg. with a ceiling of 2 gm. (0.054 gm. antimony). This analysis thus concerns 300 intravenous injections. The patients were males, 10 to 54 years of age.

Vomiting

14 of the 50 patients presented vomiting once or repeatedly in the course of the treatment.

Vomiting never occurred immediately after the injection. Thus, there is no direct drug effect on the emetic nerve centers or on the gastric mucosa. Vomiting was always of the alimentary type and stopped usually after the stomach was evacuated. (It was not possible to regulate mealtime in connection with the

injections, which preceded and followed meals at irregular intervals.) Table 2 demonstrates that vomiting is clearly an effect connected with the accumulation of the drug, becoming increasingly frequent after the last two injections of the series of six.

The 14 cases of vomiting observed during the complete course of 6 intravenous injections represent 28 per cent of the 50 cases treated. In connection with the first 5 injections vomiting occurred in 7 cases, or 14 per cent, and in connection with the first four injections only in 3 cases, or 6 per cent of the cases treated. The fact that the vomiting is related to an accumulation of the drug is further illustrated by the observation of five patients who tolerated a single massive intravenous dose of 1.0 gm. without vomiting.

TABLE 2

Number of injections preceding vomiting	1	2	3	4	5	6
Number of patients vomiting	0	1	1	1	4	7

C. *Skin*

Skin rashes occurred in 7 of the 50 patients under discussion. Morphology and pathogenesis of this phenomenon are far from being uniform.

Morphological types include:

1. Pruriginous follicular efflorescences disseminated on face, axilla, chest, extensor side of upper and lower extremities.

2. Morbilliform exanthema on face, flexor side of arms, chest, abdomen, less on lower extremities.

3. A single para-anal patch of herpetiform lesions was encountered in a female patient known to be hypersensitive to antimony and other drugs.

Table 3 indicates that the skin manifestations are connected with an accumulation of the drug, tending to appear toward the end of the treatment and several days after the treatment. All skin manifestations occurred after the 4th injection of the series of 6.

The feature common to the skin rashes observed is their transitory and benign nature. They disappear spontaneously in 2 to 5 days and in no way affect the general condition of the patient.

As to *pathogenesis*, the following factors are to be considered:

a. The morbilliform type disappeared within hours after administration of an anti-histaminic. This may indicate an allergic reaction to worms killed by the medication.

TABLE 3

	NUMBER OF INJECTIONS PRECEDING THE SKIN MANIFESTATIONS						DAY AFTER TREATMENT			
	1	2	3	4	5	6	1	2	3	4
Number of patients with skin manifestations	0	0	0	0	1	2	1	2	0	1

b. The late post-treatment rashes may be connected with the well known fact that antimony is eliminated combined with the keratin of the epidermis.

c. Prof. B. Rietman, dermatologist, Bahia, diagnosed certain skin manifestations following TWSb treatment as typical trichophytides. It would seem that massive antimony applications may revive allergic reactions to latent trichophytosis.

d. The herpetic lesion may be considered as a "breakthrough" of a latent virus.

Summing up, the majority of the skin rashes are not necessarily toxic drug effects, but appear as the result of a manifold interplay of immunological reactions.

D. *Respiratory system*

The main respiratory symptom known to complicate intravenous antimony therapy, for example with tartar emetic, is intensive and prolonged coughing immediately following the injection is apt to exhaust the patient. No such coughing occurred in connection with 360 well observed intravenous TWSb injections, including the massive dose of 1.0 gm. cited above.

Two of the group of 50 patients referred to on page 717 developed towards the end of the treatment a common cold. Although a causal relation with the treatment seems remote, it must be kept in mind that antimony may stimulate secretion in the respiratory tract.

E. *Kidneys*

In 61 cases, submitted to intensive treatment with TWSb, the urine was examined before, immediately after, and 3 weeks to 5 months after the treatment, consistently with negative findings, as to albuminuria, glucosuria and casts. In this connection it should be noted that TWSb solutions reduce Fehling's and Benedict's reagents. Positive tests for glucosuria with these reagents are only significant if checked by the fermentation method.

F. *Blood*

In the 61 cases cited in the preceding paragraph, red and white blood counts, white differential counts and hemoglobin determination were performed before, and at least once after the treatment at intervals ranging from 1 week to 2 months, in all cases without significant findings.

G. *Muscles, Articulations*

Severe arthralgias and rheumatoid myalgia are well known complications of tartar emetic treatment. Frank arthralgia was not observed in connection with TWSb treatment. One patient from the group of 50, described on page 717, complained after the 5th injection of "lumbago" and pains in the legs. No tenderness was found on pressure. Movements were in no way impeded. The symptom subsided within 3 days. Two patients mentioned after the 6th injection vague pains in the shoulders, disappearing within 2 days. Here again, the symptoms are connected with an accumulation of the drug.

H. *Local tolerance after intramuscular injection*

10 per cent aqueous solutions of TWSb were used for intramuscular treatment. The local tolerance was tested in 7 adult patients with single doses ranging from 0.1 to 0.5 gm. and total doses up to 3.5 gm., applied over a period of 11 days. This survey includes 35 intramuscular doses of 0.5 gm. In 2 cases, doses of 0.5 gm. were given daily for 5 consecutive days.

The injections caused some discomfort at the moment of the injection, vanishing within minutes. Infiltration, tenderness, impaired function or sleep did not develop in any cases. One per cent procaine added to the solution in preliminary tests proved to be unnecessary and was abandoned.

I. *Tolerance—Summary and conclusions*

Local tissue tolerance

Intramuscular applications up to 0.5 gm. TWSb, repeated up to seven times within 11 days, cause no significant tissue irritation.

Systemic tolerance

Single doses. Isolated intramuscular doses up to 0.5 gm. and intravenous doses up to 1.0 gm. (the highest single dose tested) have no untoward effect on the circulatory, gastrointestinal, nervous, cutaneous, hematopoietic systems, kidneys, muscles and joints.

Repeated intravenous doses. Intensive 2-day treatment with 6 intravenous doses applied at 8 hour intervals, totaling 0.03 to 0.04 gm./kg., with a ceiling of 2.0 gm., has no untoward effect on the circulatory, nervous, hematopoietic systems and the kidneys. In the course of this treatment benign alimentary vomiting occurred, mainly toward the end of the series. Various types of transitory benign skin rashes observed in 7 patients were mostly expressions of immunological reactions. Three patients presented mild rheumatoid pains. The majority of the untoward effects appeared during the last third of the 2 day schedule, presumably in connection with an accumulation of antimony, and therefore in all likelihood avoidable by an appropriate modification of the present treatment schedule.

As a notable and distinctive feature, the fact may be emphasized that TWSb, applied in massive intravenous doses up to an antimony equivalent of 0.7 gm. tartar emetic, lacks the hypotensive and central emetic effect of certain other oxygen- and sulphur-linked antimonials.

2. Therapeutic effects in human schistosomiasis mansoni

A. *The criteria for diagnosis and therapeutic effect* were based on the findings of living viable eggs in rectal mucosa and feces as determined by a combination of rectal biopsy findings and microscopic feces examination.

Feces examinations were performed according to the method of Hoffman, Pons and Janer (cf. Senra, J. de M., 1942). Rectal biopsies were taken in each case from four localizations, the 3 rectal valves and the ampulla. The fragments were examined fresh, compressed between slide and cover slip (Ottolina, C., 1942). This method allows for the counting of eggs with low power and the de-

termination of the stage of evolution or degeneration of the eggs with high power. Such a study is indispensable for a critical evaluation of impairments of egg production. Eggs were counted in each fragment and classified as follows:

I. *Living eggs*
   Immature: recently deposited, frequently in chains; partially developed.
   Mature: fully differentiated motile miracidia (including malformations).
II. *Dead eggs*
   Recent: granular spindleshaped, globular, split, pathological embryo, dead miracidium.
   Old: calcified, empty shells, granuloma.

The findings are reported in Table 4 in a simplified form, indicating the total number of eggs counted, the percentage of living immature, mature and dead eggs of all descriptions. The presence of immature living eggs in the rectal mucosa is an incontrovertible sign that living worms persist. The finding of only dead eggs does not exclude the presence of living worms but indicates that egg deposition is seriously impaired—whether temporarily or permanently can only be established by serial examination over at least 3 to 6 months. *The nearest goal that any therapy can and must reach is the permanent arrest of the deposition of viable eggs.* Whether all adult worms are killed or not is of secondary importance to the epidemiology of the disease.

As to the time in which a drug effect may be expected to show up on eggs and egg deposition, the fundamental data of Vogel (1942) are guiding. The evolution of freshly deposited eggs to mature miracidia takes 9 to 10 days; the life span of the miracidium in the egg is 11 to 12 days. Thus, assuming that the drug acts only on the egg in the ovary and not on the egg after its extrusion, living eggs and in particular eggs containing mature miracidia, may be expected to be found up to 22 days after an effective treatment. In other words, the presence of living mature eggs during the 3-week period following the treatment, to be referred to in the following as "induction period", does not necessarily indicate failure, but the presence of viable immature and in particular freshly deposited eggs during this period, raises considerable suspicion of failure.

B. *The results of TWSb treatment in 70 clinical cases of schistosomiasis mansoni.* Seventy male *S. mansoni* cases, 10 to 54 years of age, were hospitalized for the duration of the treatment with TWSb. The total i.v. dose ranged from 0.69 gm. to 2.3 gm., corresponding to 0.018 to 0.040 gm./kg. The total i.m. dose ranged from 3.2 gm. to 3.7 gm., corresponding to 0.050 to 0.075 gm./kg. The duration of the treatment ranged from 1 to 13 days as follows:

DURATION OF TREATMENT	ROUTE	NUMBER OF CASES
days		
1	i.v.	3
1.5	i.v.	1
2	i.v.	55
2.5	i.v.	1
3	i.v.	5
5	i.v.	2
10–13	i.m.	3

The control period, i.e., the interval between the last treatment day and the last rectal biopsy ranged from 17 to 357 days. Table 4 summarizes some representative examples of treatments and parasitological results.

Observation during the induction period (21 post treatment days).

Thirty-five cases were submitted to rectal biopsy during this period. All examinations made from the second day on revealed consistently the total absence of immature eggs, numerous before the treatment, i.e., a blocking of egg deposition.

The immediate effect of TWSb on egg deposition and development is clearly illustrated by case No. 55, Table 4. In this heavily infested patient the activity of the worms is shown by the fact that one-half of the 400 eggs counted in the rectal mucosa before treatment were living, two thirds of the living eggs being immature and one third containing fully developed miracidia. Two days after the 2-day treatment, the immature eggs completely disappeared while the percentage of mature eggs was tripled. This implies that while the deposition of new eggs ceased, the immature eggs deposited at the onset of the treatment continued their development to maturity. Twenty-seven days after the treatment, i.e., after a period exceeding the life span of the eggs, no living eggs of any description were found. The effectiveness of the treatment, i.e., the arrest of egg deposition, was confirmed 103 and 104 days after the treatment (on the 103rd day one egg was seen resembling an immature egg of doubtful viability). Dead eggs continued to be found, in decreasing numbers, over a 140 day period.

The rapid effect of TWSb on the occurrence of living eggs in the rectal mucosa is demonstrated further in cases Nos. 44 and 66. Three days after the 2-day treatment immature eggs had disappeared in both cases; mature eggs were reduced to zero in No. 44 and to one in No. 66.

Toward the end of the induction period, living but exclusively mature eggs were found in 2 cases. In case No. 9 (6 x 0.033 gm./kg. iv. in 2 days) all immature eggs had disappeared from the rectal mucosa, while a single mature egg persisted 18 days after the treatment. In case No. 14 (6 x 0.03 gm./kg. i.v. in 2 days) living eggs had completely disappeared from the rectal mucosa but were present in the feces 19 days after the treatment. Restating what has been said above in connection with the life span of the eggs, these occurrences are no proof of therapeutic failures.

On the outside fringe of the induction period the following atypical observations were made:

Case No. 23 (6 x 0.037 gm./kg. i.v. in 2 days) presented, 26 days after treatment, viable eggs in the feces but not in the rectal mucosa. After 89 days the feces were negative, but as the patient refused to submit to a further rectal biopsy the case cannot be evaluated.

Case No. 17 (7 x 0.04 gm./kg. i.v. in 5 days) is interesting as it presented, 22 days after the treatment, a single living but not "viable" egg in the mucosa, a monstrosity consisting of lumps with motile flame cells separated by irregular vacuoles. This touches on the important question—not to be discussed here—whether the drug affects the eggs exclusively in the ovary, or also after deposition in the mucosa.

TABLE 4

Therapeutic effect of TWSb in clinical schistosomiasis mansoni

Examples of individual rectal biopsy and coprological findings

PATIENT			CONTROL PERIOD (POST-TREATMENT DAYS)	RECTAL BIOPSY (EGGS)				FECES (EGGS)	TREATMENT				
				Viable		Dead	Total						
No.	age	kg.		Immat. %	Mature %	%	No.		Route	Duration days	No. injec.	Total gm.	Dose gm./kg.
16	54	53.3	0	68	11	21	147	+	i.v.	2	6	1.92	.036
			20	0	0	100	129	0					
			348	0	0	100	8	0					
44	30	60	0	+	+	++	+++	+	i.m.	13	7	3.5	.058
			3	0	0	100	51						
			45	0	0	100	8						
			64	0	0	100	2	0					
			91	0	0	100	0	0					
50	28	72.5	0	12	20	68	25	+	i.v.	2	5	1.65	.023
			19	0	0	100	3	0					
			49	0	0	100	21	0					
			82	0	0	100	22	0					
			126	0	0	100	29	0					
			258	0	0	100	11	0					
53	16	44	0	46	17	37	125	+	i.v.	2	6	1.56	.036
			18	0	0	100	6	0					
			139	0	0	100	54						
			276	0	0	100	22	0					
54	40	65	0	18	3	79	101	+	i.v.	2	6	2.0	.03
			140	0	0	100	153	0					
			307	0	0	100	47	0					
55	20	52.5	0	33	14	53	405	+	i.v.	2	6	2.1	.04
			2	0	57	43	650						
			27	0	0	100	299						
			103	0.7?	0	99.3?	134	0					
			140	0	0	100	40	0					
63	28	45.2	0	19	30	51	112	+	i.v.	2	6	1.8	.03
			19	0	0	94	15	0					
			48	0	0	100	4	0					
			67	0	0	100	3	0					
			97	0	0	100	3	0					
			134	0	0	100	2	0					
			151	0	0	100	1	0					
			207	0	0	0	0	0					
			357	0	0	0	0	0					
66	36	72.2	0	14	14	79	140	+	i.v.	3	6	2.0	.027
			3	0	3	99.7	305						
			30	0	0	100	195	0					
			138	0	0	100	102	0					
			238	0	0	100	3	0					
68	14	42.7	0	60	25	15	476	+	i.v.	2	6	1.50	.036
			22	0	6	99.4	161	0					
			264	0	0	100	4	0					
70	34	64.5	0	0	5	95	20	+	i.v.	1½	5	2.0	.031
			33	0	0	100	18	0					
			169	0	0	0	0						
			266	0	0	0	0	0					

The observations discussed in this section indicate that an intensive TWSb treatment offers the possibility of eliminating the patient as an epidemiological hazard within a period of about 3½ weeks, taking into account the life span of the eggs deposited before and up to 2 days after a 2-day course.

Recurrence of egg deposition after the induction period

Recurrence of viable eggs after the induction period, i.e., presumptive therapeutic failure, occurred in 8 cases and must be considered in connection with the dosage, as it indicates the limitations of the treatment schedule applied.

Total doses not exceeding 1.0 gm. i.v. (0.018–.03 gm./kg.) applied within 16 to 48 hours

The small doses of 1.0 gm. and less exerted in all 6 cases to which they were applied an immediate blocking effect on the egg deposition, but the 3 cases (Nos. 26; 57; 62) which were followed up by rectal biopsy for more than one month relapsed.

Cases Nos. 57 and 62, treated within a 16-hour period with only 3 injections totaling 0.69 and 1.0 gm. (0.018 gm./kg.), had negative biopsy findings after 2½ months, but viable immature eggs after 4–5 months. Case No. 62 reverted to negative biopsy findings during the 6th and 7th post-treatment months. This noteworthy observation, tending to indicate a late effect on egg deposition, remains to be evaluated by further examinations.

Case No. 26 concerns a 10-year-old boy, weighing 30 kg., treated during 2 days with a total dose of 0.93 gm., i.e., .03 gm./kg. Thirty-four days after the treatment immature and mature eggs reappeared in the rectal mucosa, while the feces were negative. It follows that the therapeutic dose is only insufficiently defined by a relative gm./kg. formula and that the total 48-hour dose i.v. must exceed 1.0 gm. to be curative. Considering that children tolerate relatively larger amounts of TWSb than adults, the dose in children can and should be increased to 0.036–0.040 gm./kg.

The foregoing observations demonstrate that small doses, under 1.0 gm./48 hours i.v., without killing the worms, profoundly damage their reproductive system. The duration of this effect remains to be studied.

Total doses from 1.1 gm. to 2.3 gm. i.v. (.023–.042 gm./kg.) applied within 1 to 3 days

Fifty-eight of the 70 cases were treated with comparable intensive intravenous dosage schedules, i.e., total doses of 1.1–2.3 gm., corresponding to .023–.042 gm./kg., applied over a period from 1 to 3 days (2 days in 51 cases, 2½ to 3 days in 7 cases). The therapeutic results obtained in this group, as defined by rectal biopsy and coprological findings, are presented in Table 5. From the 58 cases of this group, 5 relapsed (no. 31: 6 x 0.03 gm./kg. = 1.76 gm. i.v. in 2 days; no. 35: 6 x 0.03 gm./kg. = 1.5 gm. i.v. in 2 days; no. 37: 5 x 0.026 gm./kg. = 1.65 gm. i.v. in 2 days; no. 38: 6 x 0.03 gm./kg. = 1.56 gm. i.v. in 2 days; no. 61: 6 x 0.024 gm./kg. = 1.4 gm. i.v. in 2 days). The first 4 cases relapsed during the second and third, the fifth cases during the sixth post-treatment months. As this case (no. 61) had negative rectal biopsy findings on the 19th, 83d and 124th post-treatment days, the occurrence of viable eggs 6 months after the treatment is difficult to evaluate in terms of relapse or reinfection.

In case no. 31 the diagnosis of a relapse is based on the findings of a single egg, apparently viable, in a rectal biopsy performed 42 days after a 2-day treat-

TABLE 5

Therapeutic effect of intensive treatment with TWSb in 58 clinical cases of schistosomiasis mansoni

Route.....intravenous
Duration of treatment....1–3 days
Total dose.....1.1–2.3 gm (0.023–0.042 gm/kg)
Number of injections.....4–8.

CONTROL PERIOD POST TREATMENT DAYS	PATIENTS		
	treated	negative*	positive†
17–21	14	14	0
22–51	15	13	2
52–90	6	4	2
	—	—	—
First quarter year.....	35	31	4
91–120	2	2	0
121–150	5	5	0
151–180	3	2	1
	—	—	—
Second quarter year.....	10	9	1
181–210	1	1	0
210–240	4	4	0
241–270	5	5	0
	—	—	—
Third quarter year.....	10	10	0
271–300	1	1	0
301–330	1	1	0
331–360	1	1	0
	—	—	—
Fourth quarter year.....	3	3	0
Total.....	58	53 presumptive cures, 91%	5 relapses, 9%

* Absence of viable eggs in rectal biopsy and feces.
† Presence of viable eggs in rectal biopsy and/or feces.

ment. Considering the fact that a further rectal biopsy 125 days after the treatment was negative, it is doubtful whether this case should be listed as a therapeutic failure.

It appears that the total dose of 1.4 to 1.6 gm./48 hours i.v. is on the fringe of the therapeutic level and that the 48-hour intravenous dose in adults should approach 2.0 gm. as much as possible, within the range of 0.036–.04 gm./kg.

Therapeutic effect of intensive treatment observed during the 4th to 12th post-treatment month

Observations made during the 4th to 9th post-treatment month are of particular significance, because late relapses may be expected. Twenty-two out of

the 23 cases controlled 91 to 357 days after the one-to-three-day intensive treatment had negative biopsy and coprological findings (Table 5). The single relapse (or reinfection ?) observed during this period occurred during the 6th post-treatment month (No. 61; see preceding paragraph).

Therapeutic effect of semi-intensive intramuscular treatment

The 3 cases (Nos. 41, 44, 46) treated with 7 intramuscular injections (totaling 3.2–3.7 gm. = 0.05–0.057 gm./kg.) over a period from 10 to 13 days have been followed up for 2 to 3 months. No relapses occurred during this period.

Persistence of dead eggs in the rectal mucosa

The period during which dead eggs persist in the rectal mucosa is known to vary within wide limits. In case No. 63 (Table 4) the number of dead eggs decreased rapidly within 7 weeks, in case No. 66 (Table 4), slowly over 8 months. In a recent not very heavy infection, dead eggs disappeared completely within 146 days. In four cases the first completely negative biopsy findings were made 169, 207, 235 and 266 days after a 2 day treatment.

IV. SUMMARY AND CONCLUSIONS

TWSb is a water soluble trivalent organic antimonial of a new type, 50 times less toxic than tartar emetic (mouse), applicable by intramuscular and intravenous injection. In laboratory animals TWSb is therapeutically active in *Trypanosoma congolense* and *T. brucei* trypanosomiasis, in *Litmosoides carinii* filariasis and in Manson's schistosomiasis.

Seventy clinical cases of active schistosomiasis mansoni have been treated with various intensive intravenous and semi-intensive intramuscular dosage schedules. A one-to-three-day intensive treatment with TWSb has an immediate blocking effect on egg deposition. Immature eggs, numerous before treatment, were conspicuously absent in all of 35 cases submitted to rectal biopsy 2 to 21 days after treatment.

In 58 clinical cases of active schistosomiasis mansoni, TWSb permitted a safe 1-to-3-day intensive intravenous treatment with doses totaling 1.1 to 2.3 grams, resulting in 53 (91 per cent) presumptive cures, controlled up to 12 months after the treatment by rectal biopsy and feces examination. Five (9 per cent) of these 58 cases relapsed during the second to sixth post-treatment month. These 5 therapeutic failures received treatment with doses well within the tolerated range, i.e., with doses which can be safely increased.

Side effects of the intensive treatment were limited to alimentary vomiting, transitory benign skin rashes and rheumatoid pains toward the end of the treatment.

The detoxification of antimony realized in TWSb is particularly evident in electrocardiogram and blood pressure studies demonstrating that even massive doses have no significant cardiovascular effect. The following points suggest TWSb as a medication suited for the public health treatment of schistosomiasis:

the rapid treatment within 2 days;

the rapid blocking of egg deposition, probably within the first two post-treatment days;
the safety of the treatment;
the high presumptive cure rate of better than 90 per cent.

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THE VALUE OF DIFFERENT ANTIGENS IN THE DIAGNOSIS OF CHRONIC BILHARZIASIS[1] BY THE SKIN AND COMPLEMENT FIXATION TESTS[2]

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The use of the skin test and complement fixation test (C.F.T.) in the diagnosis of bilharziasis has been firmly established. The need for a rapid, sure method of diagnosis in this disease has been particularly felt in Israel where recent immigration has brought some hundreds of thousands of new residents from at least five countries of the Middle East where bilharziasis is endemic.

Skin testing was first performed by Fairley and Williams (1927) who used an intradermal injection of a saline extract of infected snail hepatopancreas while Taliaferro and Taliaferro (1931) recommended the employment of a similar extract of normal hepatopancreas as control. Antigens made from adult schistosome worms were introduced by Khalil and Hassan (1932) who made extracts of *S. bovis*, and Oliver-González and Pratt (1944) utilised *S. mansoni* for this purpose. *Fasciola hepatica* has been used as a convenient source of skin testing material as have also *F. gigantica* (Hassan and Betashe, 1934) and a number of other trematodes (Culbertson *et al.*, 1947).

Yoshimoto (1910) described a complement fixation test using as antigen an alcohol extract of adult *S. japonica* worms while Hayami and Tanaka (1910) employed an aqueous extract. Fairley (1919) introduced the alcoholic extract of hepatopancreas of snails infected with *S. mansoni* and found this antigen suitable for the diagnosis of cases of both *mansoni* and *haematobium* infection. Fairley showed that the reaction was due to the presence of a group antigen, the best extracts being obtained from snails infected with *S. spindale*[4]. Bozicevich and Hoyem (1947) made extracts of cercariae and adult worms of *S. mansoni* for use in the C.F.T. and alcoholic extracts of *F. hepatica* have also been tried (LeBas, 1923; Bettencourt and Borges, 1922). Apparently, direct comparisons between the skin test and the complement fixation tests performed on the same patients with different antigens have been made in only one series of cases (Bozicevich and Hoyem, 1947). The present paper demonstrates the efficacy of aqueous extracts of *S. mansoni* worms in the skin and complement fixation tests and their superiority to other antigens tested.

[1] The name bilharziasis is used in preference to schistosomiasis in accordance with the recommendation of the World Health Organization Expert Committee on Bilharziasis. (W.H.O. 1950).

[2] Our special thanks are due to Dr. E. Gilon for his unfailing help and advice in connexion with the clinical material.

[3] Department of Hygiene, Medical School, Hebrew University of Jerusalem.

[4] "Fairley type antigen" is here used to denote an antigen from the hepatopancreas of one or other species of snails infected with a species of schistosome, different from Fairley's original one, but prepared according to his technic.

TABLE 1

Analysis of Types of Infection in the present Series of Chronic Bilharziasis with Reference to Country of Origin

Type of Infection	Yemen	Iraq	Egypt	Morocco	Israel	Total
S. Mansoni	43	0	1	0	6	50
S. Haematobium	4	9	2	1	0	16
S. Mansoni plus S. Haematobium	4	0	0	0	0	4
Total	51	9	3	1	6	70

MATERIAL

Clinical cases

The positive series consisted of 70 hospital patients. In 64 patients the duration of the disease was at least three years; these were immigrants from four Middle Eastern countries (Table 1) who had brought the infection with them. The remaining six patients were Israelis who had been infected by bathing in the Yarkon river at least one year previously.

The ages of the patients varied from 15 to 29 years, the largest group being 20–23 years of age.

Criteria of diagnosis

Diagnosis was made by the finding of typical eggs in the stools, urine or biopsy specimens in 56 patients, and in 7 others was confirmed on the basis of a positive hatching test. The remaining 7 patients gave typical histories and clinical findings with characteristic endoscopic picture of the bladder or rectum although eggs were not found.

No difference in the reactions of patients with *S. mansoni* or *S. haematobium* infections to any of the antigens were noticed and for the purposes of the present investigation, no distinction is made between different types of bilharziasis.

Treatment

Forty-nine patients had received no previous treatment; 21 had had various amounts, from a number of injections of a schistosomicide to several full courses at different times, from several years to one month previous to the day of testing.

Control series

This series consisted of 304 persons of whom 217 were apparently healthy scientific and laboratory personnel, or persons tested during a general medical

examination; 87 were patients in hospital suffering from diseases other than bilharziasis. These included other parasitic infections, allergic diatheses, various febrile conditions, and hepatomegaly with hyperglobulinemia. Four patients had positive Wasserman reactions and one was Casoni positive. Of the persons in this series, 85 were native born Israelis, the others immigrated from 26 different countries where bilharziasis is unknown or very rare. Ages varied from 17 to 50, the largest group being 18–23 years of age. Stool and urine examinations were not performed on these subjects, except in the case of the hospital patients where schistosome eggs were at no time found.

METHODS

Skin tests

Three antigens were tested:—

Snail liver extract. A saline extract of hepatopancreas of *Australorbis glabratus* infected with *S. mansoni* (Egyptian strain) prepared according to the suggestions of Sir Neil Hamilton Fairley was supplied by Mr. O. D. Standen and was stored at 4°C. until used. The final concentration of the antigen was 1:1,000 and a parallel extract of uninfected *Australorbis* liver was used in control. In accordance with Standen's directions, 0.2 ml. was injected intradermally in the flexor surface of each forearm. A positive reaction, read after 15 minutes, was taken to be an enlargement of the initial 7–8 mm. diameter wheal to 10 mm. or more, provided that there was a difference of at least 5 mm. from the control wheal. Erythema and/or pseudopodia formation alone was not of sufficient significance and an enlargement of the test wheal to greater than 10 mm. with a difference of only 2–3 mm. from the control was taken to be a doubtful reaction.

Adult schistosome worm extract. Extracts were made in Coca's solution (0.4% phenol with 0.05% sodium bicarbonate in 0.5% sodium chloride) of worms of *S. mansoni* removed either from hamsters infected with a Yemenite strain or from mice infected with an Egyptian strain (Standen). The worms were kept in normal saline on the bench for the period of the autopsy, then briefly rinsed in distilled water and dried in a vacuum desiccator in the cold. When dry, they were ground in a mortar and preserved in the refrigerator. Latterly, batches of dry powdered worms have been preserved in sealed tubes *in vacuo*. No difference could be detected in reactions to antigens obtained from worms derived from either animal or preserved in either way.

The antigen was prepared by suspending 100 mg. of finely ground worms in 10 ml. of Coca's solution at 37°C., shaking every 10 minutes for an hour and then allowing the suspension to stand overnight at 28°C. It was then centrifuged for 20 minutes at 1,000 r.p.m. and the supernate stored as the 1:100 stock solution in the refrigerator. The pH varied from 7.35 to 7.45 and further dilutions in Coca's solution to 1:10,000 were made for use in the skin test. Bacteriological sterility of the extracts was assured by aerobic and anaerobic examination.

The test was performed by the intradermal injection of 0.1 ml. of the extract, using a 1 ml. tuberculin syringe and a 27 gauge needle with an equal quantity of Coca's solution as control. Positive reactions, read after 15 minutes, showed

enlargement of the test wheal to a diameter of 10 mm. or more with a difference of at least 3 mm. between the two wheals. In some cases, where there was erythema with enlargement to 9 mm. of the test wheal and a fading control, the result was recorded as a doubtful reaction.

Fasciola hepatica *extract.* Dry, powdered worms of *F. hepatica*, preserved since 1951, were obtained through the courtesy of Professor G. Witenberg and Mr. O. D. Standen. Extracts were made in Coca's solution as for the *S. mansoni* worms and, after a preliminary trial with extract at a dilution of 1:10,000, material at 1:5,000 was used for the tests. Performance of the test and the criteria of positivity were the same as in the case of *S. mansoni* worm extracts.

Complement fixation tests

Antigens

1. *Fairley type.* A sample of an alcoholic extract of infected hepatopancreas of *A. glabratus* infected with *S. mansoni* (Egyptian strain) prepared according to the technic of Fairley (1919) was obtained from O. D. Standen.

2. S. mansoni *worms.* The stock 1:100 extract in Coca's solution, prepared as above, was further diluted to 1:500–1:2,000 according to a preliminary titration performed on the day of the test.

3. F. hepatica *worms.* Extracts were made in the same way as for the *S. mansoni* worms and in addition, using an identical technic, extracts in absolute alcohol were made.

Technic of the test

1. *Complement titration*, using fresh pooled or commercial lyophilized complement is performed on the same day immediately prior to the test itself according to the technic of Price (1949). Two minor modifications have been introduced: first that volumes of 0.1 ml. serum are used (instead of 0.02 ml. as in the Wassermann test) and second that the titer of complement is taken to be simply that of the row containing antigen and pooled negative serum.

2. *Antigen titration.* Rising dilutions of antigen are titrated against 2, 3 and 5 M.H.D. of complement in the presence of pooled positive serum diluted 1:4. The highest dilution giving complete inhibition of hemolysis with 5 M.H.D. of complement is taken as the titer for use in the test proper. A large batch of pooled positive sera has been prepared and is stored frozen in 0.5 ml. amounts so that all batches of antigen may be tested against the same standard.

3. *Test proper.* Sera are preserved by the addition of 1:10,000 sodium merthiolate and are stored frozen until use. They are inactivated at 56°C. on the day of the test.

Each serum, diluted 1:4, is mixed with equal volumes of saline, the antigen at titer, and 2, 3 and 5 M.H.D. of complement. For the serum control, 2 M.H.D. of complement and two volumes of saline are added. In each run of tests, pooled positive and negative serum controls are included as well as negative serum with antigen and 0.5, 1.0 and 1.5 M.H.D. of complement. Fixation is performed at 37°C. for an hour and incubation continued for a further 30 minutes after adding

the sensitized cells, with vigorous shaking every 10 minutes. Readings are made immediately and checked the next morning after the tubes have stood in the refrigerator. In cases where a series of examinations are made on the same patient, all samples of his sera are examined in parallel on the same occasion.

The Fairley type antigen was examined initially by a modified method which utilised a constant amount (1.25 M.H.D.) of complement and serial doubling dilutions of the sera. This method was soon abandoned however in favor of that described, which gives more clear-cut results.

4. *Screening test.* Recently, for screening purposes, we have used a two tube test. The method is the same except that only the tube with 2 M.H.D. complement and the control is put up. Any serum giving a ± result or more, is put through the full test.

5. *Reporting of results.* 25 per cent or more inhibition of hemolysis in a particular tube is taken as a positive result for that tube. Thus, inhibition of less than 25 per cent in the first tube is reported as doubtful, greater than 25 per cent with 2 M.H.D. of complement but complete hemolysis with 3 M.H.D. is reported as +, more than 25 per cent inhibition at 3 M.H.D. but none at 5 M.H.D., ++ and so on.

RESULTS

Snail hepatopancreas antigen

Clinical cases

1. The *skin test* (see Table 2) was performed on 36 patients with proven bilharziasis; 15 were examined during the course of treatment and 17 had had previous treatment; 32 cases gave positive reactions and one, a doubtful. The reaction to the skin test was not affected by treatment.

TABLE 2

Skin & Complement Fixation Tests in cases of Chronic Bilharziasis using Infected Snail Liver Antig. Effect of Treatment

TREATMENT	SKIN TEST				COMPL. FIXAT. TEST			
	+	±	−	TOTAL	+	A.C.*	−	TOTAL
NONE	6	0	3	9	1	0	1	2
NONE PREVIOUSLY; IN COURSE OF TREATMENT	10	0	0	10	3	2	3	8
PREVIOUS COURSE(S) ONLY	11	1	0	12	6	1	3	10
PREVIOUS COURSE(S) AND IN PRESENT COURSE	5	0	0	5	3	1	0	4
TOTAL	32	1	3	36	13	4	7	24

* A.C. = Anticomplementary Serum

TABLE 3

Comparison of Skin & Complement Fixation Tests in 22 Cases of Chronic Bilharziasis, using infected Snail Liver Antigen

NUMBER OF CASES	SKIN TEST	COMPL. FIXAT. TEST
11	+	+
4	+	AC
6	+	−
1	±	+

2. *Complement fixation tests* were performed in 24 cases of whom 12 were examined during the course of treatment. Of the total, 14 had been treated previously. 13 sera were positive and 4 were anticomplementary. Insufficient data were available to draw definite conclusions as to the effect of treatment on the test (Table 2).

3. *Both tests* were performed simultaneously in 22 patients (Table 3). The skin test was the more sensitive and, unlike the C.F.T., gave no false negative results.

In 34 out of 38 cases (89%) either the skin test or the C.F.T. or both were positive.

Control cases

1. The *skin test*, performed in 33 apparently healthy people gave positive results in 9 (27%) and was doubtful in 3 (Table 8).

2. The *complement fixation test* gave one positive and three anticomplementary reactions in 8 sera (Table 8).

Reading of the results of the skin tests was made difficult by the occurrence of enlargement of the control wheal in 31 of 69 persons tested, irrespective of the existence or otherwise of specific infection. In nearly every case too, undesirable side effects manifested by erythema and pain, sometimes with swelling at the injection site, were present during the following 3–5 days. In some instances these late reactions, which were not due to bacterial infection, disappeared after 1–2 days to reappear 3–4 days later, thereafter fading slowly.

S. mansoni *worm antigen*

Clinical cases

1. *Skin testing* (Table 4) was performed in 52 patients, 7 being examined during the course of treatment and 12 having had previous treatment; 47 cases (90%) reacted positively and 1 (2%) was doubtful. Treatment had no effect on the results.

TABLE 4

Skin & Complement Fixation Tests in cases of Chronic Bilharziasis using S. MANSONI Worm Antig
Effects of Treatment

TREATMENT	SKIN TEST				COMPL. FIXATION TEST			
	+	±	-	TOTAL	+	±	-	TOTAL
NONE	30	1	2	33	24	2	1	27
NONE PREVIOUSLY; IN COURSE OF TREATMENT	5	0	1	6	10	1	2	13
PREVIOUS COURSE (S) ONLY	11	0	1	12	13	0	1	14
PREVIOUS COURSE (S) AND IN PRESENT COURSE	1	0	0	1	4	0	1	5
TOTAL CASES	47	1	4	52	51	3	5	59
TOTAL PERCENTAGES	90	2	8	100	86%	5	8%	100

2. *The complement fixation test* (Table 4), was performed on 59 sera, including 18 from patients in the course of treatment and 19 from previously treated patients; 51 (86.5%) sera were positive and 3 (5%) were doubtful. The positive cases could be divided as under +++ (21 cases), ++ (18 cases) and + (12 cases). Treatment of the bilharziasis had no clear cut effect.

In 15 patients, the C.F.T. was performed both before and (1 day to 6 months) after a full course of treatment. Thirteen patients giving a positive test before, showed no change in 9 cases, a rise in titer (1 M.H.D. complement or more) in

TABLE 5

Comparison of Skin & Complement Fixation Tests in 44 cases of Chronic Bilharziasis using S. MANSONI Worm Antigen

NUMBER OF CASES	SKIN TEST	C. F. T.
33	+	+
3	+	±
4	+	-
1	±	+
3	-	+

one, a fall in one and a serological conversion to negative in two. One patient, of two giving a negative C.F.T. before treatment, converted to positive.

3. *Both tests* (Table 5) were performed simultaneously in 44 patients. A good correlation exists between the two tests and in no one case were both negative.

Thus in a total of 67 patients, either or both tests were positive in 65 (97 %) and in the other two cases, only one of the tests was performed.

Control cases

Skin testing gave negative results in 270 (98.1 %) of 275 persons tested with doubtful reactions in 2 while the C.F.T. was negative in 296 (99 %) of 299 control subjects and doubtful in 2. In no cases were both tests positive (Table 8). With this antigen, no undesirable side effects were observed or reported in the skin test. One of the Wassermann positive sera gave a doubtful reaction while the patient with a positive Casoni test was negative. In only 5 of 351 (1.4 %) persons tested was there enlargement of the control wheal.

Comparison of snail and schistosome worm antigens

Clinical cases. Nineteen patients with bilharziasis were examined by both antigens in the skin test (Table 6) with similar results. Twenty sera were examined by the C.F.T. in parallel and here, the worm antigen gave much better results, yielding only one false negative as opposed to 7 given by the snail antigen.

Control series. In 27 healthy persons examined by both skin test, the snail liver extract gave 9 (33 %) false positive reactions and 3 (11 %) doubtful reactions while the *S. mansoni* antigen was negative in 100 per cent. Of 8 sera examined in

TABLE 6

Comparison of Antigens from Infected Snail Livers and S. MANSONI Worms in the Skin & Complement Fixation Tests in cases of Chronic Bilharziasis.

TEST	NUMBER OF CASES	SNAIL LIVER	S. MANSONI WORM
SKIN TEST 19 CASES	15	+	+
	1	±	+
	2	+	−
	1	−	+
	0	−	−
C. F. T. 20 CASES	11	+	+
	6	−	+
	1	−	−
	2	A.C.	A.C.

TABLE 7

Comparison of Antigens from S. MANSONI and F. HEPATICA Worms in the Skin Test performed on cases of Chronic Bilharziasis & Controls.

ANTIGEN	CLINICAL CASES 30			CONTROLS 36		
	+	±	−	+	±	−
S. MANSONI 1:10.000	27	0	3	0	0	36
F. HEPATICA 1:5.000	20	1	9	4	2	30

parallel, the snail antigen gave one false positive and three anticomplementary results. On the other hand the worm extract gave no positive results with any serum.

Skin tests with Fasciola hepatica *antigen and comparison with* S. mansoni *antigen*

Clinical cases. Thirty patients were tested in parallel, the *Fasciola* extract being used at a dilution of 1:5,000. Four of these patients were examined during the course of treatment and the results are given in Table 7. It is evident that with the *Fasciola* antigen alone, only two-thirds of the cases would be diagnosed, as against 90 per cent with the schistosome extract.

TABLE 8

Summary of Results of Skin & Complement Fixation Tests in 70 Cases of Chronic Bilharziasis & 304 Controls

ANTIGEN		SKIN TEST				COMPL. FIXATION TEST				
		POS	NEG	DOUBT	TOTAL	POS	NEG	DOUBT	A C	TOTAL
PATIENTS WITH CHRONIC BILHARZIASIS										
SCHISTOSOME WORM	N°	47	4	1	52	51	5	3	0	59
	%	90	8	2	100	86·5	8·5	5	–	100
INFECTED SNAIL LIVER	N°	32	3	1	36	13	7	0	4	24
	%	89	8·3	2·7	100	54·2	29·2	–	16·6	100
FASCIOLA WORM	N°	20	9	1	30	——— *				
	%	66·6	30	3·3	100					
CONTROL PATIENTS										
SCHISTOSOME WORM	N°	3	270	2	275	1	296	2	0	299
	%	1·2	98	0·8	100	0·3	99	0·6	–	100
INFECTED SNAIL LIVER	N°	9	21	3	33	1	4	0	3	8
	%	27	64	9	100	–	–	–	–	–
FASCIOLA WORM	N°	4	30	2	36	——— *				
	%	11·1	83·3	5·6	100					

* Not adequate for use in the C.F.T.

Control series. The *Fasciola* antigen gave 4 out of 36 (11%) false positive results and 2 (5.5%) doubtfuls as against a true picture given by the schistosome antigen.

Complement fixation with Fasciola *extracts*

No complement fixing antigens were detected using the "Coca extract" at dilutions of 1:2,000 to 1:100 with pooled positive sera. The alcoholic extract did fix small amounts of complement but only when tested in concentrations too high for routine use.

The results of examinations performed on a total of 70 patients and 304 controls using all three antigens are summarized in Table 8.

DISCUSSION

Skin tests

Extracts of hepatopancreas of infected snails have been widely used as skin testing material but their value is seriously limited because of the difficulty in making the readings, the high incidence of false positive results, and the undesirable side effects.

Enlargement of the control wheal in one reported series (Taliaferro and Taliaferro, 1931) was observed in 16 per cent of all persons tested and the present series gives a figure of 45 per cent. In our hands, results with this antigen are similar to those obtained by other investigators. Our criteria of positivity, however, were more liberal than those of, say, Fairley and Williams (1927). Had we accepted their criteria, nearly 60 per cent of our clinical cases would have been reported as negative but the incidence of false positive reactors would still have been high. Thus, in agreement with other workers (Mayer and Pifano, 1945; Rodrigues da Silva and Dias da Costa, 1949) we feel that this antigen should be discarded.

With respect to antigens derived from *S. mansoni* worms, the mode of preparation has been held to be of importance. Extraction of the dried, powdered worms in buffered phenol saline (Coca's solution) at 37°C. followed by overnight storage at room temperature is a convenient method. The antigen is stable and is not destroyed even by boiling for 10 minutes, (Mayer and Pifano, 1949). Extraction at low temperatures is thus unnecessary and, in any case, this procedure does not exhaust the material (rendering an effective second extraction possible) and gives an inefficient antigen (Bozicevich and Hoyem, 1947).

It has been suggested that the control injection may be dispensed with (Mayer and Pifano, 1949) but Vogel (1932) has pointed out that the Negro skin may be sensitive to phenol and we have found that 1.4 per cent of all cases, irrespective of skin color, gave a reaction to intradermal Coca's solution. We strongly recommend, therefore, the use of the control injection in every case. Using the above method of extraction, a dilution of 1:10,000 gives excellent results but it may be advisable in children, where the reaction is not so intense (W.H.O. 1953), to use 1:5,000.

Using the schistosome antigen, an enlargement of the initial wheal by 3–4 mm. or more fulfils the requirements of a positive reaction (Oliver-González and Pratt,

1944; Bozicevich and Hoyem, 1947) with a reasonable margin of error, and in our experience, more delicate criteria of positivity would make for too sensitive a test.

Fasciola hepatica antigens apart from their availability have nothing to recommend them. Our findings accord with those of most other workers (Oliver-González, 1953) as to its comparative inefficiency. Thus, only 66 per cent of our own cases would have been diagnosed by this antigen and the number of false positive reactors was high.

Of the three antigens tested by us, therefore, in the skin test, that prepared from adult *S. mansoni* worms is the best although even this may give a negative result in a clinically proved case and, exceptionally, the opposite.

Complement fixation tests

It is generally accepted that the Fairley type antigen (Fairley, 1919) gives reliable results in the C.F.T., and in addition to the original antigens prepared from different snails, extracts of the hepatopancreas of *Australorbis glabratus* infected with *S. mansoni* have yielded good material (Mayer and Pifano, 1945). In our hands however, an antigen prepared from this latter snail has not given good results. Other workers, too, in different parts of the world have found this particular antigen to be unsatisfactory (Standen, personal communication).

Except for the early Japanese investigators (Hayami and Tanaka, 1910), very few workers have used extracts of the adult schistosome as an antigen in the C.F.T. Bozicevich and Hoyem (1947) are the only recent investigators to have tried saline extracts of *S. mansoni* worms but they obtained poor results, which may have been due in part to their technic of extraction. They thus express a preference for a cercarial antigen and state that the worm antigen was anticomplementary. We, on the other hand, have never found the extract in Coca's solution to be anticomplementary at the dilution normally employed. However, the titer does drop on storage in the refrigerator and further investigations as to means of stabilizing the antigen are in progress. The preservation of the worm in 50–100 mg. amounts in the lyophilized state with the employment of fresh extracts so far holds out the best promise of maintaining a supply of material of a reasonably high titer. The presence of complement fixing antigens in alcoholic extracts of adult worms (Yoshimoto, 1910) has been confirmed, but polysaccharide fractions prepared by the method of Fuller (1938) were devoid of complement fixing antigen.

The comparatively good results obtained in the complement fixation test with *S. mansoni* material may be referred as much to the sensitivity of the test as to the suitability of the antigen. The end point was taken as only 25 per cent inhibition of hemolysis by 2 M.H.D. of complement, an increase in delicacy which was justified by the very low number (0.33 %) of false positive reactions obtained.

Using extracts of *F. hepatica* worms, Khalil (1925) claimed reasonably good results in the C.F.T. with bilharzia sera but he was obliged to exclude all Wassermann positive sera and LeBas (1923) and Bettencourt and Borges (1922) reported poor results even with alcoholic extracts. These authors found many false

positive reactors and these extracts behaved better as Wassermann than as bilharzial antigens. Of several different extracts examined, only those in alcohol showed complement fixing activity in our hands and even then in low titer.

It may be that our results, even less promising than those of others, are due in part to the employment of an old batch of worm powder, but the general consensus of opinion agrees with our rejection of *F. hepatica* worms as a source of antigen for the C.F.T. at the present time.

Treatment of the patient has an effect on his serological reactions to the *S. mansoni* antigen. In 10 of 15 cases there was no change in titer as a result of treatment, but 2 showed a rise and 3 a drop of fixation of 1 M.H.D. complement or more. With the snail liver antigen, however, it may be that treatment was responsible for the higher number of negative results in this group and Mayer and Pifano (1945) report serological conversions to negative as a result of treatment. It is noteworthy, however, that the hatching test became negative in our 15 cases, irrespective of any change in the serological picture. The skin test in our patients was not changed as a result of treatment and this accords with the views of Fairley (1952) with regard to his antigen and those of the W.H.O. Expert Committee (1953) with regard to the cercarial antigen.

Thus, by using the *S. mansoni* worm antigens described, in both the skin and complement fixing tests, a diagnosis could be made in all cases of bilharziasis so examined, a result superior to that obtained with other antigens. The efficacy of these tests is far greater than that of the usual biopsy and egg recovery technics and they should prove especially useful in the diagnosis of unisexual infections (Pifano, 1948) and in other cases where eggs are shed rarely or not at all.

SUMMARY

Extracts prepared from the hepatopancreas of infected snails and from adult worms of *S. mansoni* and *F. hepatica* were tested in skin and complement fixation tests on a total of 70 patients with chronic bilharziasis and 304 control subjects.

Skin tests with a saline extract of *S. mansoni* worms diagnosed 90 per cent of the cases and yielded only 1.2 per cent "false positives" while for the snail liver extract, the figures were 89 per cent and 27 per cent and the *F. hepatica* extract gave 66 per cent and 11 per cent respectively. The *Schistosoma* and *Fasciola* antigens were without after effects while the snail liver extract gave rise to many late and painful reactions.

For the complement fixation test, the *S. mansoni* extract also proved superior, diagnosing 86.5 per cent of cases of bilharziasis and being doubtful or positive in 1 per cent of controls. The snail liver antigen reacted positively with 54 per cent of patients' sera and *F. hepatica* extracts were without activity.

When skin tests and complement fixation tests were performed together in bilharzial patients, with the snail or with the *Schistosoma* antigens, positive results were always obtained with either or both tests.

Treatment of the chronic patients had no effect on their reaction to the skin tests with these two antigens. In the complement fixation test, no clear cut effect was observed when the *S. mansoni* antigen was used but with the snail liver

extract the proportion of negative reactors was high in a group of treated patients. Individuals who were examined both before and after treatment with the *Schistosoma* antigen showed a rise of titer in the C.F.T. in some cases, a fall in others, while in the majority there was no change.

The value of different antigens and of technics of preparation, preservation and use are critically discussed in the light of the relevant literature.

The use of an extract of *Schistosoma mansoni* worms is recommended for the skin and complement fixation tests in the diagnosis of bilharziasis.

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HUMAN FILARIASIS IN THE MARQUESAS ISLANDS[1]

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Relatively little information has been available on human filariasis (*Wuchereria bancrofti*) in the Marquesas Islands although it has been known that *Aedes polynesiensis* Marks, a vector of this parasite elsewhere in Polynesia, occurs on several of the islands of this archipelago (Marks, 1951). In June 1952 the author had the opportunity of visiting the six inhabited islands of the group for the purpose of making a brief filariasis and mosquito survey.

FINDINGS

It was quickly apparent that filariasis is endemic in the Marquesas Islands. On each of the six inhabited islands (Nukuhiva, Uahuka, Uapou, Tahuata, Hivaoa, and Fatuhiva), which together have a population of approximately 3,000 individuals, at least 5 per cent of the total population had gross manifestations of elephantiasis. On each island there were individuals with elephantiasis who had never lived elsewhere. In addition, several Europeans who had never been exposed in other endemic areas of filariasis had also acquired elephantiasis in the Marquesas Islands.

Stained blood films (20 cmm. of capillary blood) taken in the daytime from a small number of inhabitants of the island of Fatuhiva were examined and 32.2 per cent (19 of 59) were found positive for microfilariae of *W. bancrofti*. Microfilariae were found in the blood of persons who had never left the island.

A. polynesiensis was found on all six inhabited islands. It was of interest that two of these islands, Fatuhiva and Uahuka, did not have rats capable of opening coconuts. The rat-opened coconut is the most important breeding site of *A. polynesiensis* in the Society Islands. Further details of the mosquito survey of the Marquesas will be found elsewhere (Stone and Rosen, 1953).

DISCUSSION

It has generally been assumed that mosquitoes of the *scutellaris* group (which includes *A. polynesiensis*) were either present on various islands in Polynesia before the arrival of man (Buxton, 1928), or that they were carried from island to island by the Polynesians before the arrival of Europeans in the area (Davis, 1949). Moreover, the prevalence of elephantiasis among the inhabitants of certain Polynesian archipelagoes was remarked upon by several of the earliest European observers (Bennett, 1831; Lesson, 1829). In contradistinction, an

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examination of early data from the Marquesas Islands indicates that neither elephantiasis nor *scutellaris* mosquitoes existed in that area until relatively recent times.

In tracing the history of elephantiasis it is the author's opinion that more weight can be given to the lack of mention of this entity than is usually the case for less disfiguring, and hence less obvious, diseases. Likewise, the presence of mosquitoes of the *scutellaris* group is usually only too evident to those who visit areas where they occur. In the following discussion it should be borne in mind that the Marquesas Islands are grouped into what were formerly referred to as the leeward and windward islands. On certain of the leeward islands very small blood-sucking flies of the family Simuliidae are extremely annoying to man.

The earliest available data are those of Crook who spent almost two years in the Marquesas Islands (1797–1799). He made no reference to elephantiasis in his description of the endemic diseases, although he did mention such diseases as furuncles. In regard to mosquitoes he reported, "at the leeward islands they are molested by swarms of mosquitoes which have not reached to the windward part of the group" [probably Simuliidae]. In 1841, Thomson in writing of the Marquesas Islands stated, "elephantiasis so common in other parts of the Pacific is unknown here." Melville in his classic novel "Typee" (1846), which deals with his adventures on one of the leeward islands, said in regard to mosquitoes, "among the numerous afflictions which Europeans have entailed upon some of the natives of the South Seas, is the accidental introduction among them of that enemy of all repose and ruffler of even tempers—the Mosquito. At the Sandwich Islands and at two or three of the Society group there are now thriving colonies of these insects. . . . From this grievous visitation, however, the Typees are as yet wholly exempt; but its place is unfortunately in some degree supplied by the occasional presence of a minute species of fly, which, without stinging, is nevertheless productive of no little annoyance." Jardin (1858) recorded the presence of mosquitoes ("*Culex*") in the Marquesas Islands, but it is not possible to determine from his description if these were day-feeding mosquitoes of the *scutellaris* group. In 1865, in a description of the diseases of the Marquesas Islands (Anonymous), no mention was made of elephantiasis, although such diseases as typhoid, bronchitis, and asthma were included. In this same report, comment was made on the high prevalence of elephantiasis in the Society Islands. In 1877, Eyriaud des Vergnes, in a comprehensive work on the Marquesas Islands, made no mention of elephantiasis, although he did report on many other diseases such as arthritis, tuberculosis, asthma, etc. In 1880, Hercouet, in a discussion of elephantiasis in the Society Islands, stated that this disease did not occur in the Marquesas Islands. It is not until 1884 that one finds reference to elephantiasis in this area. In that year Clavel reported that "autant l'éléphantiasis des Arabes est commun aux îles de la Société, aux Samoa, aux Wallis, etc. autant il est rare aux Marquises." He went on to say that the only patient with elephantiasis whom he saw in the Marquesas was a woman with involvement of the labia majora—certainly an instance in which the etiology of the elephantiasis might be obscure.

In 1909, Dubruel stated "Il faut encore remarquer que les Iles Marquises, jusqu'ici indemnes, commencent à être contaminées depuis qu'elles sont en relation fréquente avec les Iles-Sous-le-Vent" (Society Islands). Finally, Buxton in 1928, made reference to a personal communication in which it was stated that cases of elephantiasis occur in the Marquesas Islands, although he did not supply evidence that they were autochthonous.

Several of the Marquesas Islands were sighted by Europeans in the sixteenth century, but it was not until the late eighteenth century that effective contact with the native inhabitants began to occur. Although it is not possible to state precisely when human filariasis became endemic in the archipelago, the observations cited above seem to substantiate the current reports of the local inhabitants that elephantiasis has been present only in the last 50 years. The establishment of the infection is most likely the result of the increased migration and the more rapid transportation between archipelagoes which has occurred since the beginning of European influence in Polynesia.

CONCLUSIONS

Filariasis (*Wuchereria bancrofti*) and elephantiasis are now endemic on the six inhabited Marquesas Islands. This archipelago forms the northeastern limit of the distribution of endemic filariasis and of mosquitoes of the *scutellaris* group in the Pacific Area.

Historical data indicate that both the parasite and its mosquito vector have become endemic in the Marquesas Islands since the beginning of European influence in Polynesia.

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TREATMENT OF EXPERIMENTAL TRICHINOSIS IN MICE WITH PIPERAZINE HYDROCHLORIDE[1]

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Piperazine hydrochloride has been demonstrated to be highly effective against the mouse oxyurids, *Aspicularis tetraptera* and *Syphacia obvelata* (Mouriquand, Roman and Coisnard, 1951; Standen, 1953; Brown, Chan and Hussey, 1954). Clinical trials have demonstrated the value of piperazine citrate in enterobiasis and ascariasis in man (Mouriquand *et al.*, 1951; White and Standen, 1953; Bumbalo *et. al.*, 1954; Brown and Chan, 1954; Brown and Sterman, 1954). Because of its activity against these several nematodes, its low toxicity and in view of the need for an anthelmintic in *Trichinella spiralis* infections in man, piperazine hydrochloride was tested against experimental *Trichinella* infections in mice. Our studies were designed to assess the activity of piperazine hydrochloride against the young and mature adult forms of *Trichinella* in the intestine without attempting to evaluate the effect of the drug on the larval stages of the parasite in the muscle.

Most human *Trichinella* infections are not diagnosed early, when the adult worms are maturing in the intestine, but only after the larvae begin to migrate into the muscles and produce the characteristic clinical picture. However, since the mature female worms deposit larvae for as long as six weeks, effective anti-adult therapy any time during this period would prevent further larval production and prevent additional muscle invasion and destruction. This should be beneficial to the patient and conceivably could be life saving.

MATERIALS AND METHODS

White mice, 6 to 8 weeks old, weighing from 20–30 grams, from our nematode-free colony were used in this study. In Experiment I, the larvae employed to infect the mice were obtained by digesting infected rat muscle in a pepsin-hydrochloric-acid mixture. Each mouse was given by stomach tube approximately 200 larvae suspended in gum tragacanth. On the 2nd and the 6th day of the infection, piperazine therapy was initiated at dosage rates of from 500 mg./kg. to 1,500 mg./kg. of the animal's body weight and it was continued for 7 consecutive days. In Experiment II the piperazine compound was tested against *Trichinella* during the 9th to 15th day of the infection. These mice were infected by giving each an equal portion of ground infected rat meat containing approximately 700 larvae. All the mice were autopsied 24 hours after the completion of therapy.

To ascertain the number of adult worms harbored by the treated and control mice, the small intestine of each animal was slit open in saline. Lying free in the lumen of the intestine of the untreated control mice were numerous adult *Trichi-*

[1] Acknowledgment is made to the Wellcome Research Laboratories (U.S.A.) for furnishing the piperazine hydrochloride used in this study and to Miss Sydell Kopilof for her valuable technical assistance.

nella which, according to the studies of McCoy (1932) and others, migrate out of their host as part of their normal life cycle. Relatively few worms were found free in the intestinal lumen of the treated mice, presumably because they were quickly killed by the piperazine and rapidly passed by the mice. All of these so-called free worms were enumerated, but they were not included in the assessment of the effectiveness of the therapy. Intestines of each mouse were then cut into short pieces and placed in a flask containing 25 cc. of a 0.04 per cent sodium hydroxide solution and kept at 4°C. overnight. The hydroxide solution served to dissolve the mucus of the intestine and by a vigorous shaking the following morning the worms were easily dislodged from their site among the intestinal villi into the solution, where they could be counted under a dissecting microscope (Larsh and Kent, 1949).

RESULTS AND DISCUSSIONS

Piperazine hydrochloride in the rather massive doses employed was effective against the intestinal forms of *Trichinella*, Table 1. The worm burden reduction was greater in the older infections than in the early infections. Thus, when therapy at the rate of 1,500 mg./kg. was begun 24 hours after infection, the worm reduction was 76 per cent; when therapy was initiated on the 6th and the 9th day of infection, the reduction was 87 per cent and 92 per cent respectively. The increased effect of the piperazine hydrochloride on the older infection may have been due to the natural migration of worms towards the intestinal lumen, thereby exposing themselves to the drug. At 2,000 mg./kg. daily dose, the reduction in worm count in the treated mice was 91 per cent.

In up to 1,500 mg./kg. doses, the drug regimens were well tolerated by mice; there were no signs of toxicity nor were there gross pathological changes observed at autopsy of the animals. Two of the 4 mice treated with 2,000 mg./kg. doses, however, showed diarrhea and 1 of these died on the 5th day of therapy. The LD_{50} oral dose in mice for piperazine monohydrochloride is approximately 7.6 grams per kilogram body weight.

Oliver-González and Hewitt (1947) obtained 80 per cent adult worm reduction and 99 per cent reduction of encysted larvae of *Trichinella* in rats in which treatment with 1-diethylcarbamyl-4-methylpiperazine dihydrogen citrate (Hetrazan) was initiated 24 hours after infection, in daily doses of 600 mg./kg. for 5 to 10 days. Although effective, this dose was rather large, as the oral LD_{50} of Hetrazan for rats is given as 1,380 mg./kg. Also, treatment was commenced much earlier in the infection than is usually possible in man. Magath and Thompson (1952) administered Hetrazan at a rate of 50 to 100 mg./kg. daily intraperitoneally for 2 to 3 weeks to rats, beginning at intervals of from 24 hours to over 2 months after infection. They observed that the drug did not affect the course of the disease nor was there evidence that, in rats, the piperazine compound had any demonstrable effect *in vivo* on the adults or on the migrating or encysted larvae.

Our data indicate that large doses of piperazine hydrochloride are effective against *Trichinella* in mice. Although it would not be feasible to employ such large doses in man, in view of the absence of any chemotherapeutic agent against

human trichinosis, we suggest that piperazine citrate be used in the usual therapeutic doses in a few patients to ascertain its value. Two or 3 grams daily of piperazine citrate are well tolerated, and this amount is suggested for trial in human trichinosis cases. Piperazine citrate rather than piperazine hydrochloride is suggested as it is available for human therapy.

SUMMARY

Piperazine hydrochloride in daily doses of 1,500 mg./kg. is effective against experimental trichinosis in mice. From 76 to 92 per cent of the adult worms are

TABLE 1

The effect of piperazine hydrochloride on Trichinella spiralis *in the intestine of the white mouse*

EXP.	DOSAGE RATE	DAYS OF TREATMENT	MOUSE NO.	WORMS RECOVERED FROM INTESTINAL WALL*		
				Treated mice	Controls, untreated mice	Per cent reduction
I	500 mg/kg	(7), 2-8 days of infection	1	55	136	54
			2	45	76	
			3	59	103	
			4	34	105	
I	1,000 mg/kg	(7), 2-8 days of infection	1	26	114	81
			2	17	106	
			3	21	123	
			4	18	99	
I	1,500 mg/kg	(7), 2-8 days of infection	1	22	136	76
			2	23	76	
			3	34	103	
			4	36	105	
I	1,500 mg/kg	(7), 6-12 days of infection	1	0	58	87
			2	11	80	
			3	13	41	
			4	9		
II	1,500 mg/kg	(7), 9-15 days of infection	1	7	419	92
			2	11	281	
			3	25	435	
			4	102	499	
			5	39	316	
II	2,000 mg/kg	(7), 9-15 days of infection	1	14	142	91
			2	53	478	
			3	28	332	
			4	died	393	

* Worms found free in the intestinal lumen were enumerated but not included in the figures given below.

removed by a 7-day course of the drug. It is suggested that daily doses of 2 to 3 grams of piperazine citrate be tried in human cases of trichinosis.

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TREATMENT OF *ASCARIS LUMBRICOIDES* INFECTIONS WITH PIPERAZINE CITRATE[1]

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Fayard (1949) reported on the treatment of *Ascaris lumbricoides* infections in France with piperazine (diethylenediamine). The active constituent of his syrup was piperazine hexahydrate, which is 43.33 per cent piperazine. Adults were given 1.5 grams piperazine (equivalent to 3.5 grams piperazine hexahydrate) in the evening and a similar amount the following morning. Fayard reports that approximately 2,000 patients were treated by the cooperating physicians who reported 70 to 95 per cent of their patients passed ascarids beginning usually on the second day of treatment and continuing on the following day. As many as 170 ascarids were passed by individual patients and most of the worms were alive, although dead worms were also noted. Unfortunately post-treatment stool examinations were not routinely done nor were egg counts made, and it is impossible, therefore, to determine the exact cure rate or the over-all worm reduction in this extensive series. No side-effects from the piperazine were noted.

Mouriquand, Roman and Coisnard (1951) gave piperazine to two *Ascaris*-infected children. One child, who was treated for 8 consecutive days, passed 8 worms on the eighth day and the other child, who was treated for 2 days, passed 1 worm on the second day of treatment. Post-treatment stool examinations were not reported and therefore the efficacy of the treatment cannot be assessed.

Oliver-Gonzalez, Santiago-Stevenson and Hewitt (1949) demonstrated that the complex substituted piperazine, 1-diethyl-carbamyl-4-methylpiperazine hydrochloride (Hetrazan®) is relatively effective against *Ascaris lumbricoides*, the large roundworm of man. Etteldorf and Crawford (1950), Ghanem (1954) and others have confirmed and extended these findings, and there is general agreement that Hetrazan, when given from 3 to 15 days, is effective against *Ascaris*, although its activity is somewhat irregular when given for less than 7 days.

White and Standen (1953), Bumbalo *et al.* (1954), and Brown and Chan demonstrated the effectiveness of the simple, relatively nontoxic piperazine citrate against *Enterobius vermicularis* of man, and we therefore gave it to a number of *Ascaris*-infected children to determine its activity against this prevalent parasite.

The piperazine citrate (Antepar) used in this study was supplied as an orange-flavored syrup, each cubic centimeter of which contained 100 milligrams equivalent of piperazine-hexahydrate. Twelve *Ascaris*-infected children in a rural school were given the syrup by their teacher at 8:30 a.m. when they arrived, and at

[1] Acknowledgment is made to the Wellcome Research Laboratories (U. S. A.) for furnishing the piperazine citrate (Antepar) used in this study.

3:30 p.m. when they left school. A teaspoon which held 5 cc. was given to each pupil, and by having the teacher administer the piperazine citrate we were sure that each dose was taken and that the correct amount was given. These children all received the piperazine citrate 1½ to 3 hours after the preceding meal. A group of twelve New York City children (Puerto Ricans) were given the piperazine citrate at breakfast and supper by their parents, on whom we therefore had to rely that the drug was given regularly and in the recommended amounts. One child was treated by the physician in a local children's institution for mentally defectives.

METHODS

As we had no experience with the amount of drug that would be necessary or the number of days' therapy that would be required to eliminate *Ascaris* infections, we arbitrarily chose to treat the first group of children for 7 consecutive days. The dosage of piperazine citrate was similar to that recommended for pinworm therapy:

Patient's weight	*Daily dosage of piperazine citrate (Antepar)*
15–30 lbs.	2.5 cc. (½ teaspoonful) Antepar b.i.d. = (250 mg. piperazine hexahydrate b.i.d.)
31–60 lbs.	5.0 cc. (1 teaspoonful) Antepar b.i.d. = (500 mg. piperazine hexahydrate b.i.d.)
61 lbs. and over	10 cc. (2 teaspoonfuls) Antepar b.i.d. = (1,000 mg. piperazine hexahydrate b.i.d.)

The children enjoyed the pleasant-flavored syrup and no difficulty was experienced in administering it. No special diet was suggested, there was no fasting, and no purgatives were employed before, during or following treatment.

As soon as we learned that therapy for 7 consecutive days was highly effective, we reduced the course to 5, then 4, and finally to 3 days in an effort to ascertain the minimum effective treatment schedule.

A Stoll dilution egg count was made on the stool of each patient before treatment. Two to three weeks after the completion of therapy, a zinc sulfate flotation examination was done on a stool from each child and, if *Ascaris* eggs were present, an egg count was made.

RESULTS

The assessment of the efficacy of piperazine citrate upon *Ascaris* in this study is based upon stool examinations before and after treatment. It is possible, though we think it highly improbable, that the drug may depress or stop egg production by the female worms, thus giving a false impression of its activity. However, since a number of our patients passed ascarids and since other studies indicate that 70 to 95 per cent of patients treated with piperazine passed ascarids, it seems reasonable to assume that the egg counts are a reliable index of the drug's efficacy.

Nineteen of the 23 *Ascaris*-infected individuals treated with piperazine citrate passed all of their worms as the result of a single course of treatment, Table 1.

TABLE 1

Treatment of Ascaris lumbricoides *infections with piperazine citrate (Antepar)*

NO.	AGE	WEIGHT LBS.	DAYS OF THERAPY	GRAMS DRUG PER DAY	EGG COUNT PER CC. FECES		
					Before treatment	After treatment	% reduction
1	10	58	3	1.0	8,100	500	94
2	11	52	4	1.0	11,200	0	100
3	11	116	4	2.0	3,300	0	100
4	12	71	4	2.0	9,400	0	100
5	13	77	5	2.0	11,800	0	100
6	7	47	5	1.0	2,500	0	100
7	6	54	5	1.0	16,300	0	100
8	13	66	5	2.0	2,200	0	100
9	13	114	5	2.0	4,500	0	100
10	10	77	5	2.0	8,400	0	100
11	8	66	5	2.0	7,700	0	100
12	6	48	5	1.0	8,100	0	100
13	14	87	7	1.0	36,300	400	99
14	2	35	7	1.0	7,600	2,800	73
15	12	67	7	1.0	23,300	0	100
16	14	95	7	2.0	23,300	0	100
17	1	23	7	0.25	8,100	1,200	85
18	8	56	7	1.0	12,100	0	100
19	7	47	7	1.0	4,400	0	100
20	4	52	7	1.0	2,700	0	100
21	10	65	7	2.0	100	0	100
22	16	119	7	2.0	19,600	0	100
23	4	45	7	0.5	2,600	0	100

Three children out of 11 who were given the 7-day course of therapy at home by their parents still harbored *Ascaris* several weeks after treatment. It will be noted, however, that the egg count reduction of 99 per cent was achieved in patient Number 13, whose pretreatment egg count of 36,300 per cc. of feces indicates a heavy infection, which is usually difficult to eliminate completely with a single course of any therapy. A second course of therapy completed the elimination of this child's *Ascaris* infection. The egg count of patient Number 14 was reduced by treatment from 7,600 to 2,800, 73 per cent, which may represent partial failure of the anthelmintic or failure of the child to take the therapy as directed, as this was one of the patients treated at home by the parents. Patient Number 17, a one-year-old child who was given only 0.25 gram of piperazine daily by mistake, instead of the scheduled 0.5 gram, had his egg count reduced 85 per cent. This would suggest that the daily recommended dose is approximately the required amount for successful therapy.

All of the children given the 5- or the 4-day course of piperazine citrate by their school teacher were free of the egg-passing ascarids two weeks after treatment. The only child given a 3-day course of the drug exhibited a 94 per cent reduction in egg count. The success of the 5- and 4-day therapeutic regimes given

by the school teacher, suggests that some of the failures of the 7-day treatments may have been due to failure to take the recommended course of therapy. The single patient given the 3-day treatment, whose rather light infection was reduced 94 per cent, suggests that failure may be expected with 2- or 3-day treatment regimes. This is supported by the report in Nouveautés Médicales, which states that from 70 to 95 per cent of 2,000 infected children treated with piperazine for 2 days passed ascarids. Additional studies are in progress to ascertain the effectiveness of 1, 2 and 3 days of therapy. A number of the children in our study reported passing worms fitting the description of *Ascaris*, usually on the second or third day of therapy, which suggests that the shorter treatment course may be adequate in some patients.

The minimum total amount of piperazine citrate that produced a complete cure was 0.151 grams per kilogram of weight, Patient Number 3. Patient Number 21 was given a total dose of 0.466 gm./kg. No evidence of toxicity was observed or reported by the children, their parents or the school teacher who supervised part of the study, although they were asked to keep this possibility in mind.

Clinical experience with piperazine dates back some sixty years, and for a time it was widely used in the treatment of gout and urinary lithiasis, in doses as great as 10 grams. Treatment with smaller doses was continued for weeks without reported toxicity. Hanzlik (1917), in his summary of the use of piperazine, reported side-effects such as headache, vomiting, diarrhea, urticaria, tremor, incoordination and muscular weakness. Recently piperazine citrate has been used extensively for the treatment of pinworm infection and, when used in the recommended doses, no untoward effects have been reported. However, White and Standen (1953) report side effects from the administration of piperazine hydrate in 14 adults and 6 children, consisting of giddiness, lack of coordination, difficulty in focusing vision and a sense of detachment. These effects, for the most part, were mild and recovery was rapid. These workers suggest that the side-effects were due to the drug being taken in doses considerably in excess of their recommended 50–75 mg./kg. These pinworm-infected patients received 14 days' therapy and, since it appears that *Ascaris* infections can be cured with 4 or 5 days of therapy, the likelihood of untoward effects should be minimized. All individuals treated should be asked to watch for side effects, which often are first noted only after very extensive use of a therapeutic agent.

SUMMARY

Clinical trials with piperazine citrate syrup indicate that it possesses great activity against *Ascaris lumbricoides*. The preparation is highly acceptable to children, easily administered, and requires no fasting period.

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EXPERIMENTS IN THE THERAPY OF HUMAN ASCARIASIS[1]

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In the practice of medicine in the American tropics, we have frequently been impressed by the inadequacy of the commonly accepted ascaricidal drugs and by the great need for improved ones. Therefore during the past two years, in northwestern Honduras, we have from time to time hopefully tested the effect of promising drugs against human *Ascaris lumbricoides* infections. This paper is a summary of some preliminary observations among 626 persons treated with one of six different compounds—(1) diethylcarbamazine (Hetrazan®), (2) sodium santoninate, (3) Nematolyt®, (4) hexylresorcinol monoacetate, (5) hexylresorcinol (Crystoids®), and (6) a chenopodium-chloroform mixture. Essentially, this is a report of failure to obtain outstanding results with any of these agents in the dosages so far used, although some of the cure rates are encouraging.

Our experiments were conducted in the United Fruit Company (Tela Railroad Company) hospital at La Lima, near the north coast of Honduras, and in several adjacent banana labor camps. Our aim was to determine for our area the drug which is most efficient, best tolerated, and at the same time low in cost. Because the incidence of ascariasis in this area is 30 to 40 per cent, we are particularly interested in a drug suitable for mass treatment—one that does not require administration over an extended period of time. However, several multiple-dose schedules have also been investigated.

In all instances, post-treatment fecal examinations were made 10 days after completion of treatment and again two to four weeks after treatment. Egg counts were not done. The sole criterion for cure was the complete absence of eggs from concentrated (zinc sulfate flotation) specimens on both of the post-treatment studies.

DIETHYLCARBAMAZINE

Results with diethylcarbamazine have already been reported in separate communications (Hoekenga, 1951, 1952), but are summarized here for comparison with the other drugs to be discussed. The drug was first administered, in a pilot study, to a small group of 38 Honduran adults with moderate success. No fasting or purging was employed. Nine hundred mg., when given in three divided doses on one day, resulted in 37.5 per cent cure in 8 patients; 1.0 gm., when administered in a single dose to 15 persons resulted in 32.3 per cent cure; 1.8 gm. when given in divided doses over a three day period to 15 patients gave a 60 per cent cure rate.

Following this pilot study, 125 children were treated for ascariasis with a syrup

[1] A portion of this paper was presented at the annual meeting of the American Society of Tropical Medicine and Hygiene, Louisville, Kentucky, November 12–14, 1953.

TABLE 1

*Treatment of ascariasis in children with Hetrazan® syrup**

DOSE	NUMBER TREATED	PER CENT WITH NEGATIVE STOOLS
Study No. 1		
First course: 6 mg./pound/day for four days.	95	36
Second course: original dose repeated on failures from first course.	40	30
Third course: original dose repeated on failures from second course.	20	75
Study No. 2		
12 mg./pound/day for 4 days.	30	80

* Results are based on intensive examinations done between 10 days and three weeks after completion of treatment.

containing 30 mg. of diethylcarbamazine per cc. No fasting or purging was employed. The results are shown in Table 1. Of the dosages employed, the most effective was 12 mg. per pound of body weight per day for 4 days; in 80 per cent of 30 cases, this amount resulted in absence of Ascaris eggs from the required two post-treatment examinations, and it produced no undesirable side reactions. The smaller amount of 6 mg. per pound per day gave less satisfactory results even when repeated. Eighteen mg. per pound of body weight appeared to be too toxic to the five children who received it.

It was concluded that diethylcarbamazine is a satisfactory vermifuge, particularly when administered in a palatable syrup. Its greatest use will be (1) in private practice, (2) in treatment of very young individuals, and (3) in those disease states in which fasting or purging is undesirable. However, it is not an ideal compound for mass treatment because multiple doses are necessary for good results.

SODIUM SANTONINATE

Santonin is the inner anhydride of santonic acid, whereas sodium santoninate is the sodium salt of this acid. Both santonin and sodium santoninate have ascaricidal properties, but sodium santoninate was dropped from the U. S. Pharmacopeia many years ago (United States Dispensatory, 1890) because "it is more easily absorbed than santonin in crystals, and therefore more dangerous to the patient and much less destructive to intestinal worms." Since santonin itself is quite toxic in efficient doses, this unfavorable comparison practically relegated sodium santoninate to oblivion. But presumably the structural differences of the two compounds (santonin has a lactone structure, sodium santoninate does not) have been overlooked in making this prediction of these compounds' relative toxicities. Recent work has shown quite the opposite—namely, that sodium santoninate is appreciably less toxic than santonin.

TABLE 2

*Treatment of ascariasis with sodium santoninate**

	GROUP NUMBER	PATIENTS TREATED	DOSE IN MG.	NUMBER WITH NEGATIVE STOOLS	PER CENT WITH NEGATIVE STOOLS
Single dose groups	1.	48 (adults)	130	7	14.6
	2.	44 (adults and children)	3–4 yrs..... 65 5–6 yrs..... 97 7–11 yrs.....130 over 11 yrs.....195	2	4.5
	3.	31 (adults)	260–390	9	29.0
	4.	28 (adults)	500	10	35.7
Multiple dose groups (Daily dose for six days)	5.	23 (adults and children)	4–5 yrs..... 73 7–11 yrs..... 97 over 11 yrs.....146	4	17.4
	6.	15 (adults)	250	8	53.3

* Results are based on two intensive examinations done between 10 days and three weeks after completion of treatment.

We therefore decided to retest this discarded sodium salt, and administered it to 189 individuals harboring Ascaris. We cautiously began with small single doses, and gradually increased the quantities administered. All doses were given in a flavored cascara syrup. Food was not witheld, because it is believed that the drug is absorbed more readily from the gastro-intestinal tract in the absence of food. Post-treatment examinations and criteria of cure were done as already described. The results are shown in Table 2. It is seen that with single doses of 500 mg., a cure rate of 35.7 per cent was obtained, and with 250 mg. daily for 6 days, a cure rate of 53.3 per cent was achieved.

Even though the dosage was increased by increments to quantities several times the recommended amount for santonin, there were absolutely no toxic effects noted. Sodium santoninate, therefore, merits further investigation, utilizing both higher single and higher multiple doses.

NEMATOLYT®

Nematolyt is a new German preparation containing a keratinolytic ferment, papain, obtained from the tropical melon tree (*Carica papaya*), reinforced by a suitable redox system. The ferment exerts its lytic effect on the protective keratin covering of the worm, and thus the inner organs of the worm are exposed to the destructive effect of the human intestinal ferments. The theoretical bases and first clinical experiences were published by Bohn and his associates (1949, 1950), and these observations were confirmed by several other German workers. This substance is absolutely nontoxic and large quantities can therefore be given without encountering side effects. It is important that patients receiving Nematolyt be properly prepared by eliminating protein from the diet, so that the fer-

ment acts only upon the worms. The originators of this compound claimed excellent results against *Ascaris lumbricoides* as well as against other nematodes.

We have employed Nematolyt in only eight cases. All eight had triple infections with *Ascaris*, hookworm and *Trichocephalus*. The regimen was as follows: (1) diet was almost devoid of protein on day preceding and day of treatment, (2) a saline purge was given on the night before treatment, (3) six bags of Nematolyt were administered in divided doses during the course of the day, (4) a second purge was given on the morning of the day following treatment. The results in these eight cases are as follows: *Ascaris* eggs persisted in four and disappeared in four; hookworm eggs persisted in five and disappeared in three; *Trichocephalus* eggs remained in the stools of all eight.

Although these results are not as favorable as those reported from Europe, the substance does appear to have some merit, and further investigation is indicated.

HEXYLRESORCINOL MONOACETATE

The fourth drug, hexylresorcinol monoacetate, was given a trial in Honduras because it appeared to be of very low order of toxicity and because it was of demonstrated value against ascariasis in dogs. It can be administered in liquid form or in tablets without injuring the buccal mucosa as does the parent substance, hexylresorcinol. The pilot study among 17 individuals, done in 1953, utilized dosages equivalent to the customary quantities for plain hexylresorcinol (i.e., 1.0 gm. for adults, lesser amounts for children). The results—58 per cent cures—proved to be quite misleading, for even with increased amounts of drug in 83 additional cases, the number of cures was quite unspectacular (Table 3).

However, because there was no evidence of side effects with any of these schedules, it seemed justifiable to experiment with higher single doses and with higher multiple doses of this compound. In line with this thought, six patients have received three grams daily for four days without evidence of toxicity and with "cures" in four of the six.

HEXYLRESORCINOL

Because of the unexpectedly poor results obtained with the monoacetate, we determined to re-investigate hexylresorcinol itself. In recent years, this drug,

TABLE 3

*Treatment of ascariasis with hexylresorcinol monoacetate**

	NO. TREATED	POST-TREATMENT PURGATION	ADULT DOSE IN GM.	PER CENT "CURED"
Single dose	39 adults and children	No	2.0	2.5
	18 adults	No	2.5–3.0	5.5
	7 adults	Yes	3.0	14.3
Multiple dose (daily for for 4 days)	19 adults and children	Yes	1.5	15.8
	6 adults	Yes	3.0	66.6

* Results are based on two intensive examinations done between 10 days and three weeks after completion of treatment.

with the Sharp & Dohme tradename of "Crystoids" has gained steadily in popularity. At present, it has the enviable reputation of being the anthelmintic with greatest efficiency, maximum safety and minimum inconvenience. A recent standard textbook (Craig and Faust, 1945) states that "in the average group of individuals, this therapeutic removes up to 95 per cent of all *Ascaris* worms and produces up to 90 per cent cures." Although for years hexylresorcinol has been the standard treatment for ascariasis in our hospital, it was our impression that our patients were not reacting as well as medical literature indicated that they should. Therefore we treated a group of 80 individuals with the standard single dose (i.e., one gram for adults, lesser amounts for children), and used the same criteria of cure as for the other anthelmintics being studied. The cure rate was only 42 per cent, confirming the impression. It is believed that hexylresorcinol should now be employed both in larger single doses and in multiple doses over several days in order better to ascertain its merit.

OIL OF CHENOPODIUM

Chenopodium oil, already known as an anthelmintic to the Indians at the time of Columbus, comes from the seed of *Chenopodium ambrosioides* var. *anthelminticum*. The active principle contained in the oil is Ascaridole, a substituted cyclohexane peroxide. In our comparative studies, we turned to a chenopodium mixture popularized in Central America many years ago by Rockefeller Foundation personnel. A cc. of the mixture consists of one drop each of oil of Chenopodium, chloroform, eucalyptus and menthol, in castor oil. Children are given 1.0 cc. of the mixture for each year of age up to 12 years, and adults receive 15.0 cc. (i.e., 15 drops of oil of Chenopodium). This amount is taken in the fasting state. Since castor oil is the vehicle, no additional purgation is usually necessary. Because of its simplicity and relatively low cost, this therapeutic has been used for mass treatment in our banana labor camps for many years. In this present study, the mixture was administered to 80 individuals. The result was a 40 per cent cure rate.

COMMENTS

It would seem that either the human ascarids of Honduras are particularly resistant to treatment, or else some of our therapeutic measures have been overrated. It is hoped that this presentation will stimulate critical re-evaluation of these anthelmintics in other locations.

Our criteria of cure, though simple, are stricter than those indicated in many reports. It must be remembered, of course, that many drugs will temporarily interrupt the egg-laying of the *Ascaris* worms, and if examination is done too quickly after treatment, a cure may be apparent when it is not real. In some published studies, stools were examined during the first three or four days; this is not reliable, and is a possible clue to discrepancies in results of different investigators. Even examinations one week after treatment may give misleading negative results; about 12 per cent of our cases were negative on the 10 day examination, but positive again on the two-week examination. This does not mean that additional worms were present, but that egg-laying had been resumed by female worms not expelled by the treatment.

It would have been more complete to measure reduction of worm burden by comparative egg counts before and after treatment, and by counting adult worms passed and the number of persons passing them. Unfortunately, it was not possible to carry out these procedures, although it was obvious that many persons not "cured" were being rid of a great many worms.

Possibly there would have been even fewer cures recorded if we had been able to perform egg counts, or at least done direct smears in addition to the zinc sulfate procedure. Performance of egg counts possibly would have been conducive to more exhaustive searching of slide preparations in some instances, and examination of direct smears may sometimes reveal unfertilized eggs which are passed in fairly high percentage when worm burdens become low and which oftentimes do not float on zinc sulfate.

It is also important to remember that the season of the year influences the worm burden. For the most part, all of the studies herein described were done during the rainy season—a time when the worm burden is known to be at a high level. On the other hand, the 80 per cent cure rate noted with the 12 mg./pound/day diethylcarbamazine dosage was obtained during the dry season—a time when the worm burden would be spontaneously declining. Just how much this seasonal variation in *Ascaris* egg output has influenced our apparent cure rates is not known.

SUMMARY

We have described tests with six ascaricidal compounds—diethylcarbamazine (Hetrazan®), sodium santoninate, Nematolyt®, hexylresorcinol monoacetate, hexylresorcinol and a chenopodium-chloroform mixture. None of the results are considered outstanding, but some are encouraging. Of the various single-dose treatments, hexylresorcinol gave the best results with only 42 per cent cures, the chenopodium mixture was next with 40 per cent cures, and the 500 mg. sodium santoninate dosage next with 25.7 per cent. Of the several multiple dose schedules described, diethylcarbamazine in the amount of 12 mg./pound/day for 4 days was the most promising (80 per cent cures) and sodium santoninate in the amount of 250 mg. daily for six days was next with 53.3 per cent. It would seem that in Honduras at least, there is a real need for a more efficient ascaricidal agent.

Acknowledgements. The diethylcarbamazine (Hetrazan®) used in this study was supplied by the Lederle Laboratories Division, American Cyanamid Co. The sodium santoninate was supplied by Scientific Associates. Thos. Leeming & Co., Inc. made Nematolyt® available. Strong Cobb Co. gave the supply of hexylresorcinol monoacetate. Dr. Harry Most, Professor of Preventive Medicine at New York University-Bellevue Medical Center, kindly made the arrangements for securing both Nematolyt® and hexylresorcinol monoacetate from the manufacturers of these compounds.

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INVESTIGATIONS ON EGGS OF HELMINTHS, WITH SPECIAL REFERENCE TO THE EPIDEMIOLOGY OF HYDATID DISEASE

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translated and abstracted by T. B. MAGATH

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A search for the oncospheres of *Taenia echinococcus* is basic to any study of the epidemiology of hydatidosis, but the difficulties of field surveys to find the eggs of helminths as they occur in nature in the soil, in deposited animal feces, on the fur of animals and in dirt sweepings are well known.

There are two general methods of concentrating such material to facilitate the discovery of eggs. The first consists of mixing it with water and allowing the eggs to sink to the bottom where they can be recovered by decanting or filtration. The other method is that of levitation secured by adding solutions of high specific gravity such as saturated salt solution, zinc chloride or alum, in order to float the eggs to the surface from which they can be skimmed.

A major difficulty however is in making preparations of the concentrated material for microscopic examination, since it is hard to get a sufficient amount on a glass slide, and if placed in a Petri dish the microscopic fields become less clear.

A large number of experiments were made with a view to the conservation and detection of eggs of *Taenia echinococcus*, *Hymenolepsis nana*, *Dipylidium caninum*, *Ascaris* of the pig and the dog, *Trichocephalus* and various species of *Taenia*. It was found that if several drops of the sediment, after decantation or precipitation, are placed on a piece of fine filter paper and dried carefully over a flame, paper can then be placed in a Petri dish and treated with xylol which makes the paper transparent enough to permit examination of the material through the microscope. This simple procedure achieves a further concentration of material obtained by decantation through evaporation of the suspending liquid which may amount to some 2 to 5 ml. Almost any type of paper is satisfactory and the material may be clarified with a number of organic solvents; however, xylol is the best. The preparations may be kept indefinitely and taken back to the laboratory for examination.

A method was also worked out for the fixation, staining and preservation of the material in one operation. This is done before the material is transferred to the paper. A mixture of 500 ml. of 10% formol, 50 ml. of 5% Lugol's solution, and 5 ml. of acetic acid is prepared and after treatment of the sediment with this solution, it is strained through a double layer of gauze, and alcohol is added until sedimentation of the suspended material begins. Tannin in alcohol may be substituted for the Lugol's solution and acceleration of the decantation process can be accomplished by the addition of 10 per cent of alum, which acts also as a stain fixative.

For the levitation method the author has used that of Willis which employs a saturated solution of sodium chloride. After 10 minutes the major part of the eggs float in a superficial layer and may be collected and placed on filter paper.

This method has been tested in the examination of feces from dogs, the examination of the mud washed from the belly wool of sheep and in mixtures of fecal material of animal origin, from all of which eggs of *Taenia echinococcus* and other helminths were recovered. An abundance of material is diluted with 250 to 500 ml. of water and is filtered through a metal colander on which is placed a double layer of gauze. A volume of the formula previously referred to, equal to a third of the total diluted material, is then stirred thoroughly with the suspension and alcohol is added until sedimentation begins, leaving the eggs at the surface. By addition of gelatin to solidify the entire volume of liquid material, it has been shown that virtually none of the eggs can be found on the bottom or in the middle of the mass. This method was applied to the sweepings from dirt floors contaminated with the feces of animals, and eggs of various worms have been demonstrated. The method is offered as a contribution to the study of the ecology and epidemiology of helminth infections with particular reference to hydatid disease.

THE EFFECT OF TREATMENT AND SANITATION ON HOOKWORM INFECTION IN CHOLBURI PROVINCE (CENTRAL THAILAND)[1]

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The development of quantitative methods for measuring the degree of hookworm infection makes it possible to obtain valuable information on the relative merits of certain phases of a control campaign. The study of selected groups of people before, during the course of, and after the completion of control work was carried out in Puerto Rico by Hill (1925, 1926). Sweet and his co-workers (1929) on the other hand, obtained valuable information by the examination of areas in the Republic of Panama where the history of the control measures was known even though the original situation had not been studied before the campaign began.

In order to obtain information which could be used for the establishment of permanent control measures in Thailand, a study was carried out in a selected area in Cholburi Province of the effect of treatment and sanitation on the level of hookworm infection.

DESCRIPTION OF THE LOCALITY

Cholburi Province is situated on the east side of the Gulf of Thailand about 65 miles from Bangkok. The general climatic and physiographic features of this province have been described in a previous paper (Sadun and Vajrasthira, 1953).

Two villages situated at a distance of about 15 miles northeast of Cholburi city were selected for this study (Fig. 1). One of these, Ban Serd, consists of eight communes separated from each other by rice paddies. Each commune is made up of approximately 60 dwellings. Although no recent census is available, it is estimated that about 2,500 people live in Ban Serd. The other village, Markpong, consists of nine somewhat larger communes with a total population of about 4,000 people. The two villages are separated by a canal (Ban Serd Klong). Sanitary and economic conditions in the two villages are practically the

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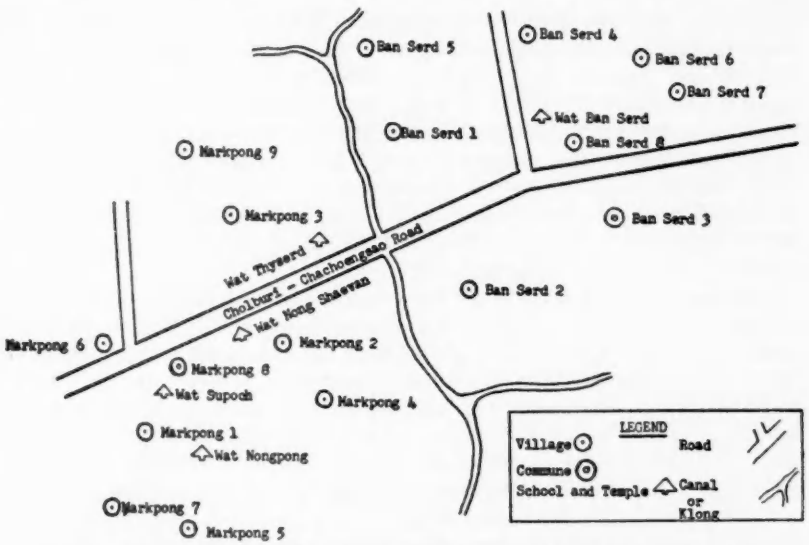

FIG. 1. Schematic map of Ban Serd and Markpong villages, Cholburi Province

same. The majority of the population is engaged in agriculture. Some men work within the village in the construction of bullock carts. In addition to their routine domestic duties, most women are engaged in weaving split bamboo baskets while others work in the rice fields.

The dwellings are built on stilts anchored on the ground. Chickens are raised by most families and are frequently seen under the houses. Some households own larger domestic animals such as water buffaloes and pigs. Rain water is the only source of safe drinking water. Nightsoil is not used as fertilizer. Pit latrines were not in existence in either village at the beginning of our study.

MATERIALS AND METHODS

From the beginning it was realized that the degree to which latrines are used in a community rather than their presence is the criterion of effectiveness in a sanitation program. A previous antihookworm campaign in Thailand (O'Brien, 1923) revealed that unless the people are convinced of the necessity of having privies, they fail to use them or keep them in repair. Even with the necessary health education it is of course not to be expected that sanitation will be perfect.

Ban Serd, Commune 3, was chosen as the first place for latrine construction since the Village Headman lived there. The need of sanitation was explained to the Headman and his cooperation was obtained in conveying the idea to the villagers. It was carefully explained that there was no wish to coerce people to build privies, no orders issued, that all expenses would be met by the villagers themselves, and that only technical advice would be provided. Most of the education was aimed at making it a source of pride, both to the individual and to the

community, that the privies were being built. With the technical advice of the Sanitary Engineer, the Headman and his friends built the first three privies. They all had a sanitary fly-proof pit, but differed greatly in type. One privy had a cement floor with a water trap, one had a wooden floor, and the third was constructed entirely of bamboo. As the program began to expand, headmen and committees from nearby communes were brought to examine the privies at Ban Serd. This helped the program at Ban Serd since it built up the feeling of pride of the villagers. The educational work was begun at the end of the rainy season in November, 1951. Most of the latrines were built at the peak of the dry season between December and February, 1952.

Stool specimens for examination were collected through the Headman of each village, or the school teachers. Arrangements were made for the people in the areas studied to deliver fresh stool specimens the morning following the day in which they received tin cans labeled with their name, age and sex. Between 70 to 80 per cent of the people who were solicited delivered specimens. Approximately one milliliter of stool was examined by the Willis-Molloy salt floatation method (1921). All specimens which were positive for hookworm were re-examined for a quantitative determination of eggs according to the modified Stoll technique (Stoll and Hauscheer, 1926). In specimens which were Willis positive and Stoll negative, the egg count was arbitrarily figured as 100 eggs per ml. of feces (Stoll, 1929). All egg counts were reduced to the basis of formed feces, and in children one to four years they were further reduced to 50 per cent because of the small size.

RESULTS

A comparison of a sanitated with an unsanitated group. As already noted, no latrines were in use in Ban Serd and Markpong villages up to December, 1951. Following an intensive campaign of health education, 181 privies were built in the eight communes in Ban Serd. The number varied from 16 in Commune 6, to 30 in Commune 5. On the average there was one latrine built for every two or three dwellings. All the communes in Markpong Village were left unsanitated in order to serve as control on the effect of sanitation. At the beginning of the experiment no noticeable difference in the incidence and severity of hookworm infection existed in the two villages. As indicated in Table 1, in February 1952, 83 per cent of 219 people examined at random (Group I) were infected with an average of 1,440 eggs per gram of formed feces (MNE, or mean number of eggs). The MNE of the total population was 1,190. One year later, 400 individuals were examined in Ban Serd where latrines had been in use between 12 and 14 months (Group III), and 400 in Markpong where no sanitation program had been organized (Group II). A perusal of Table 1 and Figure 2 shows a reduction in the per cent infectivity and intensity of infection in the sanitated village. Although much less striking, a noticeable reduction in the MNE took place also in the unsanitated control area. This may be explained by the fact that people in Markpong living in intimate contact with those of Ban Serd may have benefited by the sanitation and health education program carried out in Ban Serd.

TABLE 1

The effect of sanitation in the control of hookworm, Ban Serd and Markpong Villages

GROUP NUMBER	DATE OF SURVEY	CONTROL MEASURE	NUMBER EXAMINED	INFECTED WITH HOOKWORM			MNE OF TOTAL POPULATION
				Number	Per cent	MNE*	
I	Feb. 1952	None	219	181	83	1,440	1,190
II	Feb. 1953	None	400	348	87	930	810
III	Feb. 1953	Sanitation	400	293	73	780	570

* Mean number of eggs.

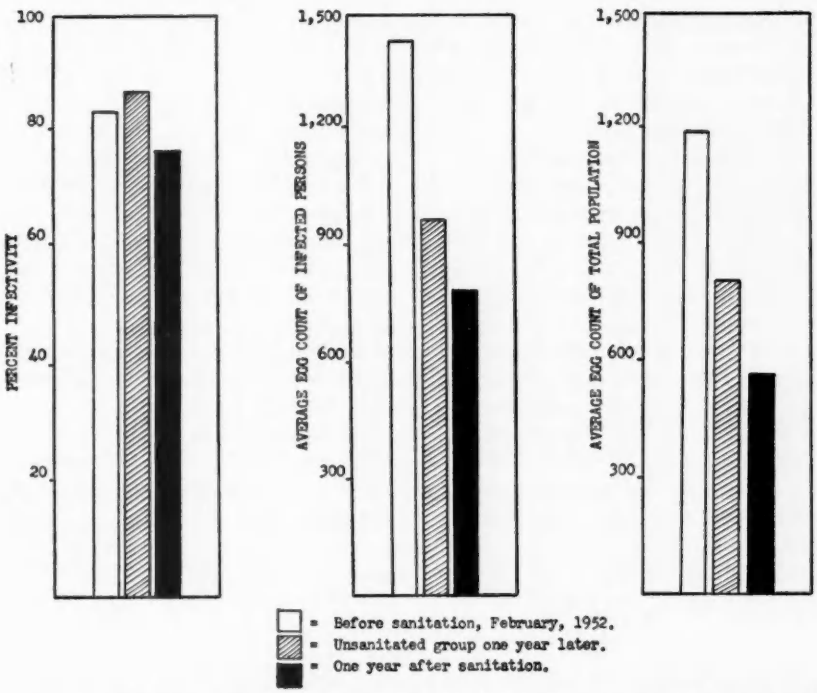

FIG. 2. A comparison of percent infectivity and intensity of infection in sanitated and unsanitated areas (Ban Serd and Markpong Villages).

The effect of treatment in sanitated group. Two communes in Ban Serd village (Ban Serd 2 and 3) were selected for the comparison of the average infection of treated and untreated individuals in sanitated areas. Only people living in houses near a latrine are included in this comparison. Treatment was given soon after the construction of latrines and consisted of a single dose of a local antihelminthic drug particularly effective against hookworm (Sadun and Vajrasthira, 1952). A total of 135 people in the two communes was examined. The people were di-

vided into two groups as nearly alike as possible with respect to the intensity of hookworm infection. Seventy-two persons were treated, and 63 left untreated as controls. Of the 72 treated persons six failed to submit their stools for examination after treatment, and 3 vomited shortly after taking the medicine. They were discarded from the experimental series. Examinations carried out six days after treatment revealed that 65 per cent of the treated persons had become free of their infection. The overall reduction of worm burden as measured by the egg count was 96 per cent. As indicated in Table 2 and Figure 3, sanitation alone (Group I), after one year failed to bring about a reduction in the number of people infected. On the other hand, the intensity of infection as measured by the MNE showed a considerable improvement. Treatment and sanitation combined (Group II) brought about a considerable reduction both in the per cent infectivity and in the intensity of infection. It can be seen that in the treated persons who lived in sanitated areas the average egg count is considerably less than in those who had not received treatment. A question may arise as to whether the considerable reduction in the worm burden among the untreated persons in Ban Serd 2 and 3 was influenced in a demonstrable way by the association with the treated persons in the same communes. A perusal of Table 3, however, indicates that the reduction in the worm burden among untreated persons in Ban Serd 2 and 3 (Group I) is not statistically different from that taking place in the other communes of Ban Serd where no treatment program was undertaken in addition to sanitation.

The intensity of hookworm infection according to different areas, sex and age groups. Egg counts of people in unsanitated areas and in areas where different control measures were applied, were divided into three categories: 0; 100 to 999; 1,000 and over. The results have been shown graphically in a series of bar diagrams (Figure 4). The large number of cases with 1,000 eggs per ml. and over, in the unsanitated areas (Groups A and B), as compared with the other series where sanitation alone, or sanitation and treatment took place (Groups C and D), is very striking.

As indicated in Table 4, the MNE for both sexes of all ages in the areas where no control measures existed was 1,120. This average count was based on 529

TABLE 2

The effect of treatment and sanitation in the control of hookworm, Ban Serd Communes 2 and 3

GROUP NUMBER	DATE OF SURVEY	CONTROL MEASURE	NUMBER EXAMINED	INFECTED WITH HOOKWORM			MNE* OF TOTAL POPULATION
				Number	Per cent	MNE	
I	Feb. 1952	None	63	49	78	1,610	1,250
II	Feb. 1952	None	63	63	100	1,720	1,720
I	Feb. 1953	Sanitation	63	56	89	670	600
II	Feb. 1953	Treatment and Sanitation	46	31	67	490	330

* Mean number of eggs.

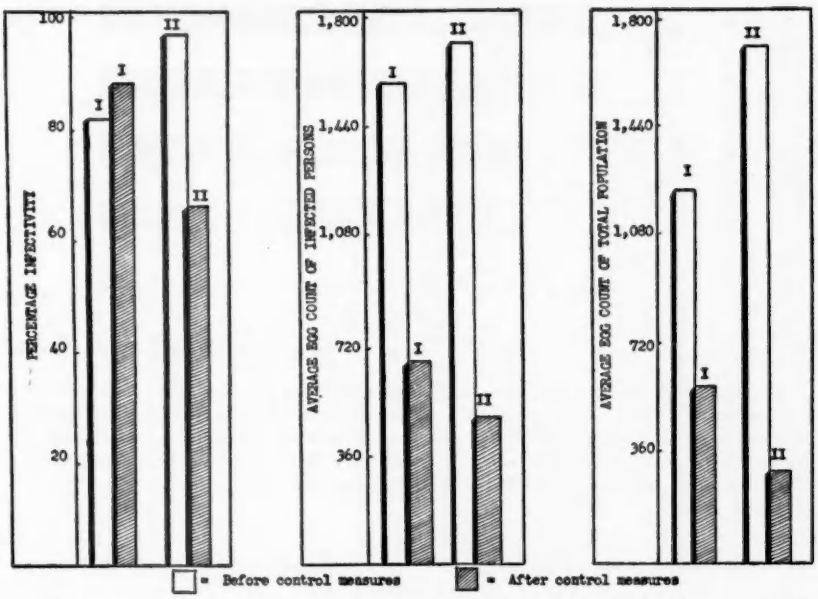

FIG. 3. A comparison of percent infectivity and intensity of infection in areas where different control measures were carried out (Ban Serd, Communes 2 and 3). Group I—Sanitation only; Group II—Treatment and Sanitation.

TABLE 3

The effect of treatment on the level of hookworm infection among untreated persons in sanitated areas

GROUP NUMBER	DATE OF SURVEY	LOCALITY	NUMBER EXAMINED	MNE* OF INFECTED PEOPLE	MNE OF TOTAL POPULATION
I	Feb. 1952	Ban Serd, Communes 2 and 3	63	1,610	1,250
II	Feb. 1952	Ban Serd, Communes 1, 4, 5, 6, 7 and 8	219	1,440	1,190
I	Feb. 1953	Ban Serd, Communes 2 and 3	63	670	600
II	Feb. 1953	Ban Serd, Communes 1, 4, 5, 6, 7 and 8	400	780	570

* Mean number of eggs.

cases. This indicates that although the incidence of infection is generally high, the severity as measured by the MNE is low when compared to the findings reported from other regions of the world where similar studies have been made.

An analysis of the hookworm infection by age groups and sex (Table 4, Figure 5) reveals that in every age group the males were more severely infected than the

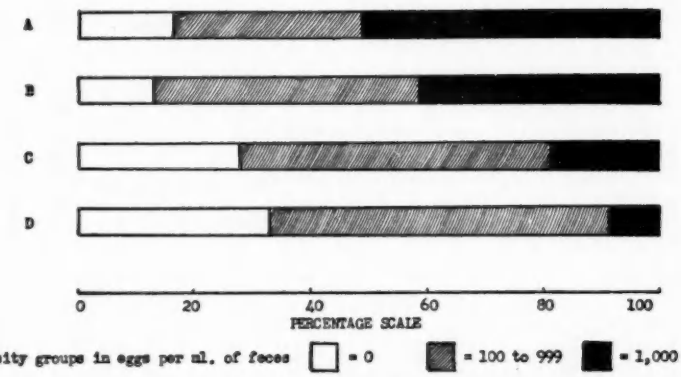

FIG. 4. A comparison of the infections in unsanitated areas and in areas where different control measures have been applied. A = Before sanitation February, 1952 (Ban Serd and Markpong villages). B = Unsanitated group one year later (Ban Serd and Markpong villages). C = One year after sanitation (Ban Serd and Markpong villages). D = One year after treatment and sanitation (Ban Serd, Communes 2 and 3).

TABLE 4

The relationship between hookworm egg counts, age groups and sex, in Ban Serd and Markpong Villages

(Exclusive of Communes where control measures were carried out)

AGE GROUPS	MALES		FEMALES		BOTH SEXES	
	Number of cases	MNE†	Number of cases	MNE	Number of cases	MNE
1–4 years	16	280*	12	150*	28	220*
5–9 years	59	1090	64	620	123	850
10–14 years	82	1100	94	840	176	960
15–29 years	33	1720	39	1160	72	1420
30+ years	51	1960	79	1390	130	1610
All ages	241	1310	288	960	529	1120

* These figures show a 50% reduction of counts for stool size in children 1 to 4 years old.
† Mean number of eggs.

females. This relationship between the two sexes has often been found in various studies (Carr, 1926; Hill, 1927; Sadun and Vajrasthira, 1953) where differences in habits bring males more frequently in contact with polluted areas. This is particularly true in this region where most women stay around the houses where they are engaged in the weaving of split bamboo baskets, in addition to the household duties and rearing of children. Most males, on the other hand, are engaged in agricultural practices, thus spending part of the day in the fields where pollution is more common. A perusal of Table 4 and Figure 5 also reveals in both sexes a gradual increase in the severity of infection with age up to adulthood.

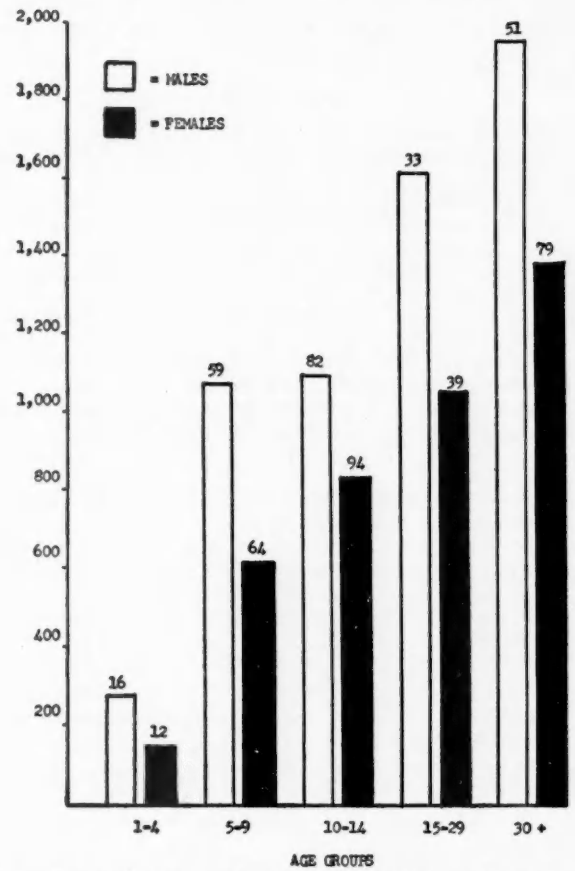

FIG. 5. Comparison of the egg counts of hookworm in the unsanitated areas for age and sex. The numbers above the bars represent the number of cases upon which the average count is based.

DISCUSSION AND SUMMARY

The results of this study, in general, indicate that a significant reduction of the worm burden is already evident one year after the establishment of control measures. When sanitation constituted the only control measure, the average intensity of infection decreased. Best results were obtained, however, whenever treatment and sanitation were carried out in the same place. No studies on the effect of treatment alone have been carried out since no further evidence is needed of the ineffectiveness of treating a portion of the population without sanitation other than for its educational value and temporary individual relief.

This study indicates that continued sanitation and mass treatment represent

the best methods to reduce hookworm infection in Thailand to the point where it will cease to be a public health problem.

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CORRESPONDENCE

April 29, 1954

To the Editor:

The American Foundation for Tropical Medicine would like to bring to the attention of the readers of the JOURNAL openings at the Liberian Institute, Harbel, Liberia, for a parasitologist with a medical degree, a malariologist, and laboratory technician, with experience in Parasitology.

The Liberian Institute of the American Foundation for Tropical Medicine, Inc., is a research and training center in tropical medicine located in Harbel, Liberia, West Africa, and operated by The American Foundation for Tropical Medicine, Inc. It was established in 1950 and consists of a large, two story, two-wing, laboratory building with 24 individual lab rooms, three two-bedroom residential houses, and a four-bedroom guest house with joint dining and kitchen facilities. The laboratory is furnished with modern lab furniture equipped with gas, vacuum, water, and electrical outlets. It has cold storage and deep-freeze rooms and is mechanically complete. The laboratory, residential houses, and guest house are of brick and re-enforced concrete carefully designed to meet the conditions of a tropical climate. Additional residential houses are to be constructed later.

Liberia has an ideal tropical climate and those who live there enjoy it. The Institute is located two miles from Roberts Field, international airport for West Africa and five miles from the Firestone Plantations Company.

As you know, Dr. Max J. Miller, former Associate Professor of Parasitology at Macdonald College, McGill University, accepted the position of Research Director of the Liberian Institute early in 1953; and on April first of that year, took up his duties in Liberia. During his year's tenure Dr. Miller has initiated studies in malaria control, schistosomiasis, trypanosomiasis, onchocerciasis, and filariasis.

A parasitologist, malariologist, and laboratory technician are needed to assist Dr. Miller in his broad program of research in tropical medicine. Persons interested in applying may obtain application forms from: Mrs. Herma E. Hoefler, The American Foundation for Tropical Medicine, 345 Madison Avenue, New York 17, New York. We welcome questions regarding the positions, the Liberian Institute, and other phases of our work and interests.

Sincerely yours,

John Abbink, *President*
The American Foundation
for Tropical Medicine

BOOKS RECEIVED

Histology. Edited by Roy O. Greep, Ph.D., Dean and Professor of Dental Science, Harvard School of Dental Medicine, with thirteen contributors. 953 pp., 648 illustrations, with many in color. New York and Toronto: The Blakiston Company, Inc. Price $15.00.

Expert Committee on Drugs Liable to Produce Addiction. Fourth report. WHO Technical Report Series No. 76. 12 pp. Geneva: World Health Organization. 1954. Price 1/9, Sw. fr. 1, $.25.

Expert Committee on Poliomyelitis. First report. Technical Report Series No. 81. 69 pp. Geneva: World Health Organization. 1954. Distributed by Columbia University Press, International Documents Service, 2960 Broadway, New York 27, New York. Price $.50, 3/6, Sw. fr. 2.

Expert Committee on Alcohol. First report. WHO Technical Report Series No. 84. 16 pp. Geneva: World Health Organization. 1954. Price 1/9, Sw. fr. 1.—, $.25.

The Mentally Subnormal Child. Report of a Joint Expert Committee convened by WHO with the participation of United Nations, ILO, and UNESCO. WHO Technical Report Series No. 75. 46 pp. Geneva: World Health Organization. Distributed by Columbia University Press, International Documents Service, 2960 Broadway, New York 27, New York. Price 1/9, $.25, Sw. fr. 1.—.

Expert Committee on Environmental Sanitation. Third report. WHO Technical Report Series No. 77. 25 pp. Geneva: World Health Organization. 1954. Price 1/9, $.25, Sw. fr. 1.—.

Expert Committee on Rheumatic Diseases. First report. WHO Technical Report Series No. 78. 25 pp. Geneva: World Health Organization. 1954. Distributed by Columbia University Press, International Documents Service, 2960 Broadway, New York 27, New York. Price 1/9, $.25, Sw. fr. 1.—.

Expert Committee on Rabies. Second report. WHO Technical Report Series No. 82. 27 pp. Geneva: World Health Organization. 1954. Price 1/9, $.25, Sw. fr. 1.—.

Histopathologic Technic and Practical Histochemistry. By R. D. Lillie, A.B., M.D. Medical Director, U. S. Public Health Service; Chief, Pathologic Anatomy Service, Clinical Center, National Institutes of Health; and Chief, Laboratory of Pathology and Pharmacology, National Institute for Arthritis and Metabolic Diseases. 456 pp. New York & Toronto: The Blakiston Company, Inc. 1954. Price $7.50.

Treponematoses, a World Problem. By T. Guthe & R. R. Willcox. 79 pp., illustrated. Geneva: World Health Organization. 1954. Distributed by Columbia University Press, International Documents Service, 2960 Broadway, New York 27, New York. Price 3/6, $.50, Sw. fr. 2.—.

Smallpox Vaccination, a Survey of Recent Legislation. 41 pp. Geneva: World Health Organization. 1954. Price 3/6, $.50, Sw. fr. 2.—.

The Science Book of Wonder Drugs. By Donald G. Cooley. 247 pp. New York: Franklin Watts, Inc. 1954. Price $2.95.

The Meaning of Social Medicine. By Iago Galdston. 137 pp. Cambridge: Harvard University Press. 1954. Price $2.75.

Das Subaquale Darmbad und seine Verwendung. By Gottlieb Olpp. 191 pp. Stuttgart: Hippokrates-Verlag Marquardt & Cie. 1954. Price DM 14.50.

A Manual of Tropical Medicine. By Thomas T. Mackie, M.D., Col., M.C., A.U.S. (Retired); George W. Hunter, III, Ph.D., Col., M.S.C., U.S.A.; C. Brooke Worth, M.D. 2nd ed. 907 pp.; 304 illustrations with 7 in color. Philadelphia and London: W. B. Saunders. 1954. Price $12.00.

Handbook of Tropical Dermatology and Medical Mycology. Vol. II, including separately bound Appendix on Fundamental Dermatological Terminology. By R. D. G. Ph. Simons (ed.), Senior Lecturer at the Dermatological Clinic of the University of Leyden, and 58 Collaborators. 1705 pp. Amsterdam, Houston, New York, London: Elsevier Press, 1953. Price $15.00.

BOOK REVIEWS

Advances in the Control of Zoonoses. Bovine Tuberculosis, Brucellosis, Leptospirosis, Q Fever, Rabies. WHO/FAO Seminar on Zoonoses, Vienna, November 1952. WHO Monograph Series #19. 276 pp. Geneva: WHO. Distributed by Columbia University Press, International Documents Service, 2960 Broadway, New York 27, New York. French edition in preparation. Also published as FAO Agricultural Studies #25. Price: $3.00, 15/-, Fr. fr. 960.-, Sw. fr. 12.-.

The prevention, control and, for some, the ultimate eradication of zoonoses in human beings is the goal of the World Health Organization and the Food and Agriculture Organization of the United Nations. The principal papers and summaries of discussions presented at a seminar in Vienna form the content of the monograph. It deals with bovine tuberculosis, brucellosis, leptospirosis, Q fever and rabies from an administrative point of view and presents useful summaries on recognized laboratory diagnostic methods. The well-documented chapter on the epidemiology, diagnosis and prophylaxis deserves general recognition on account of its balanced appraisal of the available knowledge. Every one of the papers is informative, and their content must be considered the latest authoritative information on the the five most important zoonoses. As a source of reference and quick orientation it is equally useful to the physician, public health worker and veterinarian. Laboratory workers would benefit greatly by following the simple, but explicit, description of the principles and procedures proven dependable by experienced investigators. This monograph must be available to those who have direct or indirect contact with domesticated or wild animals. Although it discusses certain diseases of public health and economic importance in Europe, the general conclusions and recommendations are applicable to every inhabited region of the world.

K. F. MEYER

Bacterial Genetics, by WERNER BRAUN, Ph.D. 238 pp., illustrated, with 67 fig. Philadelphia & London, W. B. Saunders Co., 1953, $6.50.

To whom is this book directed? The author states, ". . . the text endeavors to furnish a general account of the present status of bacterial genetics in a manner which, it is hoped, is elementary enough for the beginning student and sufficiently comprehensive to be of value to the research worker" (presumably in bacteriology). Firstly, inasmuch as genetic principles pervade all of the biological sciences including bacteriology, the reviewer seriously doubts that there is any substitute for a course in the principles of classical genetics for the beginning bacteriology student today. The 15 per cent of the text devoted to a review of genetic principles is necessarily rather sketchy and inadequately illustrated. Further, the specialized field of bacterial genetics cannot reasonably be divorced from the general subject of microbial genetics for either the beginning student or the research worker. As a consequence of the restriction of examples to bacteria, certain aspects are necessarily incompletely developed. Secondly, Braun pleads a need for the book on the grounds that fundamental contributions are widely scattered in journals not read by bacteriologists. However, an analysis of the references shows that over one-half are in bacteriological journals. Many of the significant papers have been compiled recently by Lederberg and reprinted by The University of Wisconsin Press, 1952. Thirdly, for the research worker the book is of questionable value as much of the text is loosely written, incompletely documented, and noncritical. For example, in discussing the limitations of the lyophil technique Braun states, ". . . it has been observed with bacteria as well as with fungi that after reconstitution of apparently homogeneous lyophilized cultures a small percentage of previously unr detected variants may be present. . . . Experimental studies have indicated that the effect is not due to selective killing." The reference to fungi concerns results obtained aftelyophilizing mixtures of mutant yeast cells (not homogeneous cultures) and the data certainly are not conclusive. The reference to bacteria concerns Braun's own research on

Brucella in which no data are presented and which concludes that lyophilizing can produce changes due to selective killing.

The section on population changes is well presented. Since bacterial genetics is a new and active field, it is not surprising that the author falls short of his broad objectives.

R. W. BARRATT

The Anopheline Mosquitoes of the Indo-Australian Region, by J. BONNE-WEPSTER, M.D. in collaboration with N. W. SWELLENGREBEL, Ph.D. From the Department of Tropical Hygiene and Geographical Pathology of the Royal Tropical Institute, Amsterdam. 504 pp. 176 figures. J. H. de Bussy, 1953.

A total of 114 species and subspecies are considered in this extensive review of the anopheline mosquitoes of the Indo-Australian region. Following a section on morphology and techniques, descriptions are given of the adult, male terminalia, and larva of each of the species and subspecies, except for those stages which have not as yet been described. This is almost entirely a review of the literature; many descriptions are taken directly from other publications and this apparently is true of most of the illustrations. In addition there are brief summaries of the bionomics, distribution, and relation to malaria of each of the species. There are 22 distribution maps. At the end of the book keys are given to adult females and fourth stage larvae.

Although this is a conventional taxonomic treatise, it is gratifying to note that the authors recognize the limitations of a purely morphological approach to the solution of problems of speciation among the many closely related anopheline populations of this region. They make no claim that all of the forms described are necessarily distinct species. Their objective apparently was simply to present all of the descriptive information now available. A great deal of work has been done in recent years on the anophelines of this region, and the review performs a useful function in bringing together all of this information. It is unfortunate that Reid's (1953) revision of the *A. hyrcanus* Group could not be included.

LLOYD E. ROZEBOOM

Map of World Distribution of Rickettsial Diseases, Tick-borne and Mite-borne Forms. (No. 11 of the series in preparation of an Atlas of Diseases). By Department of Medical Geography, American Geographical Society. New York, American Geographical Society, 1954, Price $1.25 folded, $1.50 flat.

Like other maps of this series dealing with infectious diseases, this one, concerned with the distribution of the tick-borne and mite-borne forms of rickettsial diseases, represents a highly useful contribution to the practicing epidemiologist, to the teacher of epidemiology and to others concerned with rickettsial diseases. The preparation of the map obviously involved collation and integration of a tremendous amount of literature.

If, therefore, an occasional error is uncovered, it is understandable if one comprehends the magnitude of the task undertaken in the preparation of these plates. Consequently, to raise objections is, to some extent, merely to cavil. Thus, to indicate the occurrence of two different diseases within a geographic area, a single color is used. Perhaps this is, on the whole, justifiable, but it may, in some cases at least, be misleading. For example, the Pacific Coast of the United States is indicated as being an area of both tick-borne typhus and of Q fever. The reader cannot tell which areas are affected by Q fever, which by tick-borne typhus and which by both diseases. Speaking of Q fever, one might question some of the statements made concerning the epidemiology of this disease. While numerous species of ticks in various parts of the world have been found to be naturally infected, all the available evidence indicates that the tick plays a relatively unimportant or minor role in the transmission of the disease to man. Inhalation of the rickettsia is believed to be the most common mode of infection, and the common source of the rickettsia is not from the dry feces of ticks as stated but from the afterbirth and birth fluids of livestock harboring the rickettsia.

Nevertheless, the American Geographical Society has rendered an invaluable service in producing these maps, and we are indebted to them for a difficult undertaking which has been so creditably and so remarkably well accomplished.

EDWIN H. LENNETTE

Modern Concepts of Communicable Disease, by MORRIS GREENBERG, M.D., M.S.P.H., Director, Bureau of Preventable Diseases, New York City Department of Health; Assistant Professor of Epidemiology, Columbia University, and ANNA V. MATZ, R. N., M.A., Public Health Nursing Consultant in Communicable Diseases, New York City Department of Health. Foreword by Harry S. Mustard, M.D. 563 pp. with illustrations. New York: G. P. Putnam's Sons, 1953. Price $6.20.

The authors have attempted to illustrate the tendency toward integration and synthesis of modern preventive medicine, curative medicine, social medicine, and public health in the narrow but appropriate field of communicable disease.

The approach is through a rapid historical summary of concepts: the rise and development of bacteriology and immunology, the accompanying science of sanitary engineering, the discovery of antibiotics and the results of this current emphasis on the last of the epidemiologic triad (host, environment, agent) to receive frontal attack. Changes in control measures to keep pace with newer knowledge are succinctly outlined. For example, with reference to the almost obsolete practice of placarding, the authors say "We have not observed the placard to do more than scare away delivery boys. The discarding of placards by the New York City Department of Health has not arrested the downward trend of communicable disease in the city." Or this, on nursing: "Furthermore, the elaborate nursing procedures adopted in former years, the mystic rite of the basin of smelly disinfectant into which the nurse carefully dipped her fingers before leaving the room, the sprinkling of disinfectant solutions on walls and floors, the hanging up of sheets which had been soaked in dilute carbolic solution have all been shown to be elaborate mumbo jumbo. Nursing in communicable diseases is essentially the same as nursing in other diseases." The movement toward disbanding special hospitals for communicable diseases and opening up general hospitals for their care under certain safeguards is emphasized by devoting a well written chapter to "Hospital Management of Communicable Diseases," in addition to a discussion of home care which brings out the ultimate responsibility of the family for nursing care, and the role of the professional nurse as guide and assistant. Rather elementary discussions of "Infection, Resistance, and Allergy," methods of treatment of communicable disease, medical asepsis, epidemiology, and community control (local, state, national) are also included in "Part I—The Basic Principles of Communicable Diseases."

In the second part of the book, the specific diseases are classified according to their major mode of transmission: respiratory, food- and water-borne, those transmitted by contact and by arthropods. The discussion of each disease covers its agent, host, and environment. Medical care plans are presented, which include treatment, diet, and supportive nursing care.

Unfortunately, considerable repetition seems necessary when a broad, systematic, and categorical discussion outline is followed for each disease. Further, in some of the diseases, it appeared difficult to fill in the details of the outline, while for others, it appeared difficult to reduce the discussion to proportion for the book. This may result in the student-reader perhaps giving more priority to the present importance of some of these diseases than is warranted.

The intent to classify the diseases by mode of transmission is not clearly labelled in the Table of Contents or stated in the Preface. There are those who might argue against the inclusion of Poliomyelitis and Infectious Hepatitis under Respiratory Diseases in the light of current work.

Miscellaneous statements in the text appeared to this reviewer to reveal either uneven coverage of the current literature or hurried editing. There are rather more spelling and grammatical errors than I had expected to find, especially in the cited references, even in a

first-edition. The size of the volume is convenient, the typography clear, and the illustrations excellent. It should be most useful to nursing students, certain graduate public health students, and public health workers as a text, and possibly helpful to the general medical practitioner for quick review and reference.

ARTHUR C. HOLLISTER, JR.

Gesundheitsbüchlein für die Tropen, by ERNST VON HALLER, M.D., Lower Buchanan, Grand Bassa County, Liberia, with foreword by Professor E. G. Nauck, Director of the Bernhard-Nocht-Institut für Schiffs- und Tropen-Krankheiten. 92 pp. Paper covers. Stuttgart O., Georg Thieme Verlag. 1951. Price DM 5.70.

Here is a pocket-size booklet, written in German, full of wise and useful information for the layman who is called on to live in the Tropics. Well-written by a good observer and an experienced physician, it deals, in five sections, with the climate, adaptation and general risk of disease in warm climates. A description of measures concerned with living conditions, nutrition, housing, servants and the hygienic rules to guide the care of children is followed by an account of preventive measures against infections and diseases in general. The briefing of the most important diseases encountered in the Tropics is excellent and absolutely up to date. This guide to healthful living around the Equator, which includes a good index, will prove most useful and is highly recommended.

K. F. MEYER

Eleven Blue Men And Other Narratives of Medical Detection, by BERTON ROUECHÉ, 215 pp. Boston, Mass. Little Brown & Co., 1954. Price $3.50.

These twelve short stories of detective work in the realm of disease are highly recommended to physicians, health officers and laymen alike. They are not only written in a lively and skillful way with all the interest and suspense of mystery tales, but they are well and truly told with a simplified scientific approach. They all appeared in the *New Yorker* between 1947 and 1953 and aroused interested comment in medical circles; now they are in book form. They deal with actual problems of diagnosis and epidemiology, many of which got into the papers and which were solved by a combination of intelligence and fortuitous circumstance, just as in detective fiction. There are in addition well written little essays on the smog problem and on the intense search now going on for antibiotics. The book will give the layman an insight into the modern machinery of disease investigation and control, and the medical man will find epidemiology treated from an unusual angle. Mr. Roueché received a Lasker Foundation Award for medical reporting in 1950 for some of these stories.

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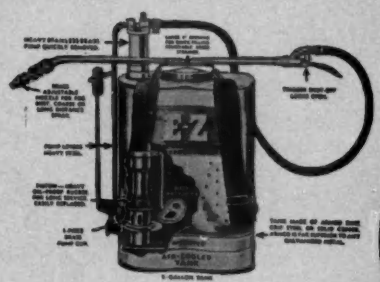

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